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TABLE OF CONTENTS

No. 1, March, 1945

Paper		Page
271	The relation of cell size and organ size to mortality in Habrobracon By Daniel Swartwood Grosch	1
272	The effects of thiourea on amphibian development By Albert S. Gordon, E. D. Goldsmith, and Harry A. Charipper	19
273	Experimental study on the rôle of trephocytes in regeneration of Eisenia foetida By Emil Liebman	43
274	Seasonal changes in Obelia colony composition By Frederick S. Hammett and Dorothy W. Hammett	55

No. 2, June, 1945

Paper		Page
275	Tolerance of larvae of Drosophila for amino acids: Tryptophane By Louise Palmer Wilson	145
276	A study of inducement and transplantability of sarcomata in rats By Helen Dean King and Margaret Reed Lewis	155
277	The effects of carcinogens on growth of fungus hyphae By Elwyn Reese and Lois Reese	177
278	The relation of cell division to growth rate in cucurbit fruits By Edmund W. Sinnott	189
279	A modified Helianthus test: I. Comparative elongation rates employing two methods of applying the growth factors By John C. Fardon, Sr. Mary Thoma Maynard, O.P., and Sr. M. Margaret Ann McDowell, O.P.	195
280	A modified Helianthus test: II. The effect of pH in certain media on the zone of cell elongation in the hypocotyl of Helianthus annuus By John C. Fardon, Sr. Mary Thoma Maynard, O.P., and Sr. M. Margaret Ann McDowell, O.P.	207
281	A modified Helianthus test: III. The effect of different concentrations of indole-3-acetic acid on cell elongation in the hypocotyl of Helianthus annuus seedlings By John C. Fardon, Sr. Mary Thoma Maynard, O.P., and Sr. M. Margaret Ann McDowell, O.P.	217

No. 3, September, 1945

Paper		Page
282	Studies on the growth-promoting factor of adult tissue extracts: III. Precipitation with alcohol; IV. Action of lipid solvents By L. Doljanski and H. Werner	229
283	Growth processes of Corallorhiza By Howard S. Reed and Daniel T. MacDougal	235
284	A note on the action of the bar series in Drosophila By Richard B. Goldschmidt	259
285	The deciduous dentition of the chimpanzees By Henry W. Nissen and Austin H. Riesen	265
286	Some effects of hexenolactone on tissue cultures By Jane G. Royle	275

Book Review

Nutrition and Glands in Relation to Cancer. By F. E. Chidester. (Reviewed by Frederick S. Hammett) 285

No. 4, December, 1945

Paper		Page
287	A note on the rate of morphogenetic movement in the slime mold, Dictyostelium discoideum . . . . . By John T. Bonner and Donald Eldredge	287
288	Graduation of human growth curves . . . . . By G. A. Baker	299
289	Relative growth in races of mice produced by selection . . . . By John W. MacArthur and Leo P. Chiasson	303
290	The rate of growth of tadpoles in varying concentrations of Ringer's solution . . . . . By William Etkin and Alvin Seligson	317
291	Prepubertal castration in the male chimpanzee with some effects of replacement therapy . . . . . By George Clark	327
292	Tolerance of larvae of Drosophila for amino acids: tyrosine, phenylalanine, and alanine . . . . . By Louise Palmer Wilson	341
293	Integrated programs for Growth Symposia . . . . . By Frederick S. Hammett	353

Book Review

Bioenergetics and Growth. By Samuel Brody. (Reviewed by Frederick S. Hammett) 357

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THE RELATION OF CELL SIZE AND ORGAN SIZE TO MORTALITY IN HABROBRACON

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(Received for publication on November 2, 1944)

Among animals, insects of the order Hymenoptera provide especially favorable material for a study of the relationship between chromosome number and the size of the organism and of its component parts because males, developing from unfertilized eggs, are haploid. This was recognized by Nachtsheim (1913) and by Oehninger (1913) who compared cells of the haploid drone honeybee with those of the diploid female worker and demonstrated that, in this case, cells with fewer chromosomes were comparable in size to cells with more chromosomes. Prior to this study the widely accepted concept had chromosome number the chief factor in determining cell size.

The present paper deals with the relationships between cell size, sex types, and differential mortality in the parasitic wasp *Habrobracon juglandis* (Ashmead). Habrobracon is particularly favorable for studies of this kind because in addition to the parthenogenetically produced haploid males, a diploid male type can be derived from fertilized eggs. A series of allelic factors occurs designated xa, xb, xc, etc. (Whiting 1943). Haploid males may have any one of these factors, diploid males are homozygous and females are heterozygous for any combination of two. Diploid males appear if the sex allele of the haploid male parent is similar to one of the pairs of sex alleles present in the diploid female parent. Thus when only two sex alleles are involved in the cross (xa/xb female by xa male for example), three classes of offspring are produced: (*a*) biparental diploid females, xa/xb; (*b*) impaternate haploid males, xa and xb; and (*c*) biparental diploid males, xa/xa. Were it not for differential mortality, diploid males should be numerically equal to their sisters.

It has been shown (Speicher, 1934; Risman, 1941) that diploid males have much larger cells than diploid females, while haploid males have cells approaching the diploid female size. It seemed possible

that large cell size might be responsible for the high diploid male mortality and that a demonstrable difference in cell size might appear between diploid males differing in mortality. For this reason the present investigation was especially directed toward a comparison of cell size in groups of diploid males from different stocks.

A series of crosses known to produce diploid males was made between various stocks. Three of the combinations were finally chosen producing offspring in which there was high, low, and intermediate mortality of diploid males respectively. In the three classes of offspring from each combination, measurements were made of the entire animal, of certain of its structures, and of the cells from which these structures were formed. Resulting data, supplementing those of Speicher and Risman, give additional evidence on the cell size relationships of diploid males, diploid females, and haploid males. The data present a basis of comparison of cell and structural size in animals with contrasting mortality and show that cell size may be important in producing differences in male mortality.

Thanks for help and guidance are expressed to Dr. P. W. Whiting under whose direction the present investigation was carried out.

Materials and Methods

The three types of crosses made for the present study involved six inbred Habrobracon stocks. Especial care was taken that stocks contained no tendency toward aberrations which might affect structure or cell size. Three of the stocks had recessive eye colors to serve as differentiators between haploid and diploid males. Each of these stocks had sex alleles corresponding to those of a wild type stock used. Females with recessive eye colors were mated to wild type haploid males of the same sex allele system. Diploid male offspring, being biparental, had wild type eyes.

Females of mutant stock 25-c with "cantaloup" eyes were crossed with males of wild type stock 25. From preliminary observations it seemed probable that the offspring, called *ai* material, since sex alleles involved were xa and xi, would show low diploid male mortality. The second type of cross was made between females of mutant stock xg/xh-wh with white eyes and wild type males of stock 1. It was expected that the offspring, called *gh* material from the sex alleles xg and xh, would show high diploid male mortality. The third type of

cross was between females of mutant stock with orange eyes, xe/xf-o, and wild type males of stock 33. It seemed probable that the offspring, called *ef* material from the sex alleles xe and xf, would show intermediate diploid male mortality.

The offspring from each cross were examined; the three classes of offspring (females, haploid males, and diploid males) were counted and preserved in alcohol. Wasps which seemed undernourished were discarded. Sometimes larvae are shaken off the food host before they have developed to full size. In such a case, the larva pupates but develops into a dwarf imago. Only extremely dwarfed individuals were discarded to avoid selection against genetically determined dwarfism. Therefore, although it is a matter of chance whether diploid male, haploid male, or female larvae fall off the host caterpillar, if a certain amount of dwarfness were characteristic of normal haploid males extreme types would not have been preserved and are not considered in these data.

Breeding was continued until at least one hundred diploid males were obtained from each combination (Table 1). This resulted in the general examination of more than 14,405 animals. From these were selected at random for study one hundred wasps of each of the three types of offspring from each of the three combinations, thus totalling 900 specimens.

1. *Size of Cells on Wing Surface*

In developing insects it was observed that each bristle on a wing surface corresponds to and gives indication of the presence of a wing surface cell (Dobzhansky, 1929; Speicher, 1934). Since each cell of the single layer forming the upper surface of the wing bears a single bristle (microchaeta), variations in cell size produce proportional variation in dispersal of bristles. Thus, if fewer microchaetae are noted per unit area larger cells are present in the wing surface.

For the present investigation of cell size, the right primary wings of the wasps were removed, transferred to xylol, and mounted in damar on a glass slide. The number of bristles (microchaetae) on a determined area of the upper surface of each wing was recorded by the Dobzhansky method. In this procedure paper is ruled into squares, equivalent, under the magnification used, to a desired surface area of the wing. By aid of a camera lucida the position of the

microchaetae in the limited area is noted. The region examined is chosen in a part of the wing least subject to modification or wrinkling. This region was that adjoining and distal to the stigma. Because bristles have unequal spacing near veins, the limits of the surface area chosen were not near veins. Since starvation or unfavorable temperature may change insect cell size and therefore influence bristle pattern (Alpatov, 1930) all wasps were fed adequately and incubated at constant optimum temperature.

2. *Length of Animal*

The length of each wasp was measured with an ocular micrometer. The anterior surface of the head and the posterior tip of the abdomen (not including the ovipositer of the female) were taken as the limits of measurement. All animals were well preserved in an extended position.

3. *Wing Size*

In order to have a basis of comparison of wing size, the vein which extends along the greater part of the costal margin of the wing was measured with the aid of an ocular micrometer. This long wing vein was selected because it is stiff, not liable to be injured or distorted in the development of the animal or in the technique of examination.

4. *Eye Facet Size*

An insect eye facet is secreted by two cells lying side by side beneath it. Bridges (1925) considers facet size an accurate index of the size of the cells composing the ommatidium.

A camera lucida was used in measuring facet size in the right compound eye of each wasp. A high power ocular (30x) was employed to provide adequate magnification with a 16 mm. objective. This relatively low power objective was necessary because it has a working distance great enough to permit adequate illumination of the object by reflected light. An objective with a smaller working distance throws a shadow on the object. With this lens system, three consecutive facets were the greatest number that could be measured without introducing the factor of eye curvature. Total width of three facets, amounting to diameter summation, was measured after camera lucida projection.

5. *Eye Size*

The eye of a wasp is a rounded projection from the side of the head. Determination of the surface area of a structure of this shape would be technically very difficult. In the present investigation, instead of representing eye size by surface area, the plane enclosed by the line delimiting the eye from the head was used as an index of eye size. This outline, termed the "base" of the eye, is elliptical. The length (major axis) and width (minor axis) of this elliptical outline were measured with an ocular micrometer. The plane within the elliptical outline was then calculated by multiplying the product of the major and minor semiaxes of the figure by π.

6. *Antennal Flagellum Length and Segment Number*

The flagellum of the right antenna of each wasp was examined. Length was measured with an ocular micrometer and number of segments was counted.

Results

1. *Ratios in Offspring of Selected Crosses*

In dealing with the offspring of a cross it has been found useful to take the more viable, stable female as a basis for mortality comparison. Ratios of haploid males to females and of diploid males to females can then be calculated. If ratio of either diploid males or haploid males to females is low, the mortality rate is high. Ratios of males to females resulting from the three combinations selected for investigation, are shown in Table 1. In *ai* material (25-c x 25)

TABLE 1

Total Offspring Obtained from Three Types of Crosses Excluding Haploid Males Produced in Vials Subsequent to Exhaustion of Mother's Sperm Supply

	Stock 25-cantaloup ♀♀ by stock 25 wild type ♂♂ (*ai* material)	Stock xe/xf-orange ♀♀ by stock 33 wild type ♂♂ (*ef* material)	Stock xg/xh white ♀♀ by stock 1 wild type ♂♂ (*gh* material)
Diploid males	153	149	108
Haploid males	207	1,290	4,342
Females	383	1,334	6,439
Number of crosses	12	54	176
Ratio $\frac{\text{haploid males}}{\text{females}}$	0.5407± 0.0204	0.9670± 0.0035	0.6743± 0.0045
Ratio $\frac{\text{diploid males}}{\text{females}}$	0.3992± 0.0211	0.1116± 0.0082	0.0167± 0.0015

the ratio for diploid males was high as compared with the other material, while the ratio for haploid males was low. In *gh* material (xg/xh-wh x 1) the ratio for diploid males was very low and the ratio for haploid males was intermediate. The extremely low ratio of 0.0167 for *gh* diploid males is a condition of special interest. A low ratio was expected but in no crosses of this type was such an extreme anticipated. In *ef* material (xe/xf-o x 33) the ratio for diploid males was intermediate and the ratio for haploid males was high. From these results it is evident that a ratio for one kind of males is not predictable on the basis of the other ratio.

2. *Microchaetal Counts*

In Table 2 are shown the results of counting microchaetae in a unit area of wing surface. The tabulated values indicate that diploid males had fewer microchaetae per unit area, therefore larger cells, than females or haploid males. In all material these differences are statistically significant and furnish additional evidence that diploid male cells are larger than female cells. Also in Table 2 is presented additional evidence that haploid male cells closely approach the size of female cells.

TABLE 2

MEAN NUMBERS OF MICROCHAETAE PER 0.01 SQUARE MILLIMETER OF WING SURFACE IN RADIAL CELL

	ai material	*ef* material	*gh* material
Diploid males	9.62±0.07	9.35±0.08	9.27±0.10
Haploid males	16.33±0.10	14.41±0.11	13.14±0.12
Females	15.57±0.10	14.09±0.11	13.56±0.13

The data furnish a basis for comparison between sex types with low mortality and comparable forms with high mortality. Table 2 shows that in *ai* material microchaetae are more numerous, therefore cell size smaller, than in comparable forms of *ef* or *gh* material. In *gh* material cell size is greater than in *ef* material but for the diploid males the difference is not significant.

Cell size tendencies are represented graphically in Figure 1 in which frequency distribution of animals is plotted against number of microchaetae per unit area of wing surface. The curves of haploid males and females cover approximately the same ranges except that

haploid values as a group tend to be shifted toward the upper or small-cell end of the scale. The significant parts of the curves representing diploid male values are alone in the lower end of the scale and scarcely overlap the haploid male and female range. The curves of these latter, more viable types are asymmetrical with a definite tailing off of distribution toward the upper end of the scale. Skew for these curves is positive being 0.69, 0.35, and 0.97 for the haploid males and 0.51, 0.87 and 0.69 for the females of *ai*, *ef* and *gh* materials respectively. Curves for diploid male values show tendency away from such positive skew, being 0.63 and 0.27 for *ef* and *gh* respectively and actually negative, —0.77, for *ai*. Thus gentle tailing toward the upper end of the scale is not typical of curves for diploid male values and in this these curves differ from the curves of more viable types. Another manner in which curves for diploid male values differ from curves representing values of more viable types is by starting abruptly at relatively high frequency at the lower end.

It is also evident that curves for diploid male values differ from those of other classes of offspring in kurtosis. In each kind of material the peak for diploid male values is higher than the peaks of the curves of the two other classes which indicates higher degree of concentration about a point of maximum frequency. If curves for diploid males from different material are compared, peaks are lower in the order of increased mortality. Therefore when material with higher mortality is sampled, there is greater dispersion within the same range of values. The bearing of this statistic and graphic evidence on male mortality is presented in the discussion.

3. *Length of Animal*

The mean body length for one hundred animals from each of the three types of offspring in each combination of sex alleles was calculated. No statistically significant differences were found between diploid males and females while haploid males were consistently shorter. Since the mean values of the different sex types were comparable, results can be grouped without regard for sex alleles. Thus, in the conditions under which the animals were reared, mean length for the total 300 diploid males was 2.846 ± 0.009 mm.; for haploid males, 2.797 ± 0.009 mm.; for females, 2.852 ± 0.009 mm.

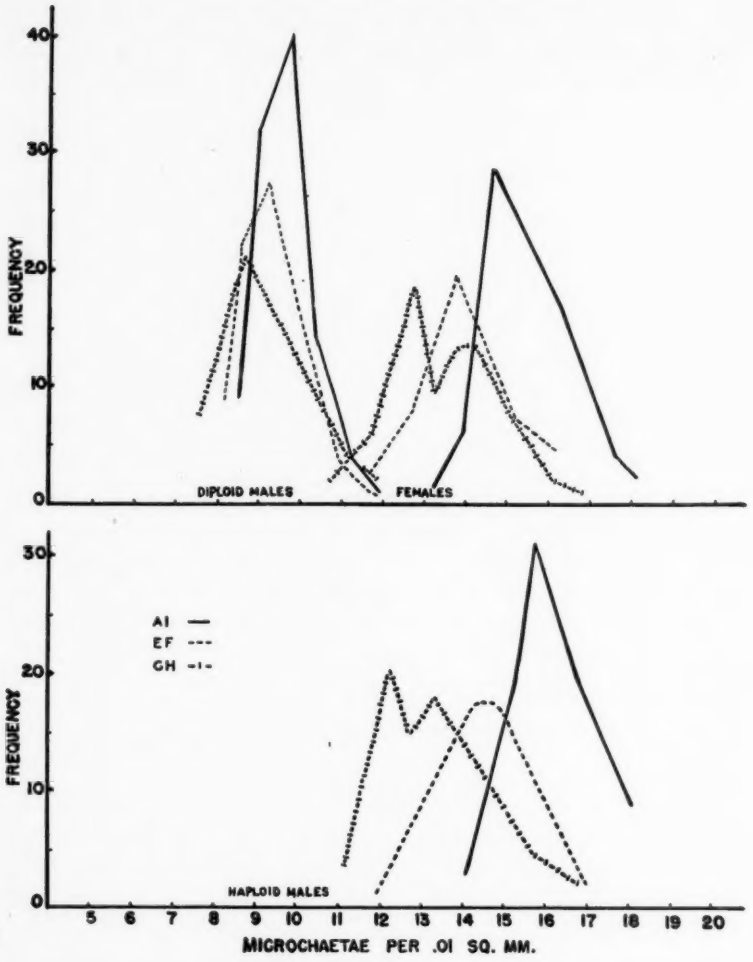

FIGURE 1

Frequency arrays of microchaetae per 0.01 square millimeter of radial cell (Rower and Gahan) of right primary wings from diploid males, haploid males, and females from offspring of crosses between inbred stocks with sex alleles xa/xi (*ai* material), with xe/xf (*ef* material) and with xg/xh (*gh* material). The arrays include counts from 100 specimens of each of the nine types (three sex types from each of the three types of crosses). Average area covered by epidermal cell may be obtained in square micra by dividing 10,000 by the number of microchaetae corresponding to any given frequency.

4. *Length of "Costal" Vein of Wing*

Comparison of measurements of vein along costal margin of the right primary wing showed a situation similar to that of measurements of length of total animal. Mean lengths for diploid males and females were similar, while means for haploid males were lower. Again, if results be grouped according to sex type of animal, mean lengths for "costal" veins were 0.981±0.005 mm. for diploid males, 0.958±0.005 mm. for haploid males and 0.978±0.003 mm. for females.

5. *Eye Facet Size*

Table 3 represents the mean widths of diameter summation of three eye facets in different types of offspring in *ai*, *ef*, and *gh* material. This eye facet evidence on cell size supplements that from microchaetae counts. Diploid male values for width of three consecutive ommatidia are consistently greater than female or haploid male values. However, differences between the above three types

TABLE 3

MEAN WIDTH OF THREE EYE FACETS IN MILLIMETERS

	ai material	*ef* material	*gh* material
Diploid males	0.0412± 0.0002	0.0399± 0.0002	0.0406± 0.0002
Haploid males	0.0367± 0.0002	0.0362± 0.0002	0.0364± 0.0002
Females	0.0362± 0.0002	0.0361± 0.0002	0.0368± 0.0002

of offspring are not as significant as those of microchaetae counts and no significant differences are shown between comparable classes of offspring except that diploid males of *ai* material have larger facets than diploid males of *ef*. Since the cells producing eye facets can vary in three dimensions, the measurements of one dimension should not be expected to give an impressive demonstration of cell size difference. Certainly differences are not as striking as in microchaetae counts which represent two dimensional aspect of a variation which is chiefly two dimensional.

6. *Eye Size*

Mean value of areas calculated for the "base" outline of compound eyes are presented in Table 4. Male eyes are larger than female eyes.

TABLE 4

MEAN AREA OF "BASE" OF COMPOUND EYE IN SQUARE MILLIMETERS

	ai material	*ef* material	*gh* material
Diploid males	0.0525± 0.0004	0.0520± 0.0003	0.0512± 0.0007
Haploid males	0.0537± 0.0001	0.0502± 0.0003	0.0499± 0.0004
Females	0.0449± 0.0002	0.0474± 0.0003	0.0472± 0.0002

This tendency is dependent on sex rather than cell size since cell size of haploid males closely approaches that of females.

7. *Antennal Length and Segment Number*

Table 5 shows mean values of antennal flagellum length. It is evident that the shortness of female flagella is a very obvious characteristic of the sex. It is a well-known difference and is easily seen

TABLE 5

MEAN LENGTH OF ANTENNAL FLAGELLUM IN MILLIMETERS

	ai material	*ef* material	*gh* material
Diploid males	1.778± 0.008	1.748± 0.008	1.770± 0.015
Haploid males	1.777± 0.013	1.762± 0.001	1.794± 0.008
Females	1.075± 0.003	1.042± 0.005	1.019± 0.005

with the unaided eye. Diploid male antennae prove to be as long as haploid antennae in these data with no significant differences demonstrable.

The data furnish a basis for comparing flagella length in equivalent male types of *ai*, *ef* or *gh* offspring. No differences significant in relation to cell size and mortality are demonstrable between comparable offspring of different origin.

As shown in Table 6 male antennal flagella have more segments than female flagella in each type of material. In the discussion a mathematical consideration is presented which indicates that, although length of antennal segments is slightly less in females than in males (Table 7), the shortness of female antennae is chiefly due

TABLE 6

MEAN NUMBER OF SEGMENTS PER ANTENNAL FLAGELLUM

	ai material	*ef* material	*gh* material
Diploid males	17.28±0.06	16.74±0.06	18.13±0.12
Haploid males	18.93±0.04	18.97±0.05	19.21±0.06
Females	12.10±0.03	11.89±0.03	12.02±0.01

to their low segment number. Table 6 also shows that in each type of material mean number of segments is lower for diploid males than for haploid males. Thus, although male antennae are of comparable length, the diploid has fewer but longer segments (Table 7).

TABLE 7

MEAN LENGTH OF ONE ANTENNAL SEGMENT IN MILLIMETERS

	ai material	*ef* material	*gh* material
Diploid males	0.1066± 0.0003	0.1023± 0.0004	0.0966± 0.0006
Haploid males	0.0941± 0.0004	0.0919± 0.0003	0.0928± 0.0003
Females	0.0892± 0.0002	0.0868± 0.0003	0.0855± 0.0004

DISCUSSION

1. *Diploid Male Mortality*

In the present data the extremely low ratio of diploid males in *gh* material demonstrates how important mortality can be in producing deviation from the expected ratio. Some unfavorable developmental condition restricting the number of adult diploid males must be very active in *gh* material, a situation on which investigation of mortality influence should be most productive. The present investigation considered cell size in this low ratio material and in materials with contrasting high and intermediate diploid male ratios. However, animals examined were adults that had managed to survive unfavorable developmental conditions; through death, extreme deviates were probably elimniated from materials investigated. Evidence from mean values and graphed frequency distribution of microchaetae data suggests that cell size is larger in diploid male material which has

higher mortality. Presumably contrasts in cell size would be much more striking if cell sizes could be compared for embryonic stages which would include both viable and inviable types.

Graphed frequency distributions of michrochaetae counts seem to show influence of mortality on the arrays of diploid male cell size. Curves for diploid male values tend to have relatively slight skew with values clustered around a mode from which frequency distribution falls off rapidly. Toward the small-cell end of the scale there is tailing off although slight, while in the larger-cell end of the scale curves representing diploid male values start abruptly without tailing off. Individuals with extremely large cells were not observed. Such animals would have completed the curve and made it more symmetrical. It is assumed that mortality accounts for this complete absence of adult diploid males with cell size above that of the clustered group.

Large size of diploid male cells as compared with female and haploid male cells has proven to be such a consistent characteristic that next to diploid chromosome content, it is the most typical cytological and basic structural quality of the diploid male. Present in diploid male cells is a surface-volume relationship unlike that of the cells of more viable types. Perhaps mere mechanical difficulties arise in the physiology of diploid male cells or possibly doubled gene conditions which can obtain in a homozygous diploid male might function poorly under this surface-volume relationship.

According to present theory determiners of sex differences in Habrobracon may be part of a differential chromosome segment which acts as the sex allele. Consisting of many genes determining the structural, functional, and behavioristic sex differences characterizing Hymenoptera, a gene system of this kind could in the aggregate have duplicate effects such that all haploids or homozygotes are approximately similar and male. A diploid male, through its homozygous condition of the sex allele would be under the influence of double "doses" of determinants of mortality, as well as other differences, although genic balance would be undisturbed. This condition acting in the environment of large diploid male cell size might be important in influencing diploid male viability.

2. *Diploid Male Cell Size*

In Habrobracon, cell size regulation results in haploid male cells which are comparable in size to diploid female cells. There is also cell size "regulation" in the diploid male where cells relatively larger than those of the female are produced. It seems possible that large diploid male cells are the result of increased activity of the regulator responsible for large haploid cells. Since the regulator is active in the diploid male which is homozygous for the sex allele and is not active in the female which is heterozygous for the sex allele, it appears that genes responsible for the regulator, or in the activity of the regulator, must be part of the differential chromosome segment which acts as a sex allele. Moreover, it seems that doubling of these genes through the homozygous condition of the sex allele may be an important factor in increasing the activity of the cell size regulator in the diploid male.

3. *Size of Body Structure in Types of Offspring*

Consideration of gigantism has been an attendant phase of cell size investigation from the beginning of work with animal polyploids. Most of the classic cell size literature contains brief mention of the subject. General, though not complete, reviews of the field, appear in Artom (1928) and Fankhauser (1941). It has been shown that gigantism is not always evident in general body dimensions of polyploid animals and that in many animals gigantism is not typical for polyploid individuals. Observations on diploid male Habrobracon in the course of the present study indicate that gigantism is not obvious in the diploid male in spite of large cell size. The comparable body and wing vein lengths of diploid males and females supply statistical evidence that gigantism is not typical for what might be considered an example of polyploidism, the diploid male. Since individual cell size is greater, some regulator of cell number must operate early in the development of the individual so that the diploid male is made up of fewer but larger cells.

The haploid male, the usual type in the Hymenoptera, has entered even more into discussion of body size than the diploid male. Drone honey bees were examined by Nachtsheim (1921) and proved to have a body size range overlapping that of the diploid female worker. In view of their slightly smaller body cells, haploid males might be

expected to exhibit a tendency toward dwarfism. Such a size difference is apparent between haploid male tissue and female tissue in Habrobracon gynandromorphs (Whiting, 1932a). In such animals male and female tissues, as parts of a single individual, are under identical environmental influence of temperature, nutrition, and population. Any slight contrast which develops in these exactly comparable conditions is readily apparent with tissues side by side. In view of the tendency toward smaller size of haploid tissue in gynandromophs, it is interesting to note in the present study how means of body length and of "costal" vein length tend to be smaller for normal haploid males than for other types of offspring.

4. *Structural Size as a Secondary Sex Character*

Secondary sex characters are dependent upon difference in ratio of male-producing to female-producing genes rather than upon chromosome number. In applying this genic balance principle to Habrobracon, dominance relationships of the elements within the sex alleles must of course be considered. Thus if the elements within xa be expressed as Fg and within xb as fG, then either Fg, Fg/Fg, fG, or fG/fG would be male-producing while Fg/fG would be female-producing. Influence of the recessive genes or gene groups designated f and g would be negatived by the dominant female-producing genes F and G.

Difference between males and females in antennal length and in eye size are to be regarded as secondary sex characters. Eye size difference is not as clear cut and evident as antennal length. That male eyes are larger has been observed in gynanders (Whiting, 1932b). The present data provide statistical evidence on this point.

An analysis of the influences responsible for overall length is difficult because they are complex and one is never sure that unknown factors of residual heredity are identical. Complexity is apparent when the opposing tendencies of number and size of segments are considered. Diploid males and haploid males may have antennae of comparable length (Table 5) yet diploid male antennae are made up of fewer segments (Table 6). Diploid male antennae then have longer segments. This tendency can be represented mathematically by dividing antennal length by number of segments. The necessary assumption, which overlooks the tapered tip segment, considers all

segments to be of equal length. Table 7 presents these calculations and shows statistically the consistently greater length of diploid male antennal segments. This might be considered an influence of large diploid male cell size and additional evidence that diploid males have large cells.

Table 7 also indicates that the shorter length of female antennae is due primarily to small number of segments, since mean length per antennal segment, although slightly less, is shown to approach that of the male. If the number of such segments were as high as that of the male, on the basis of length one would be unable to distinguish female antennae without measuring them.

In view of this situation in the female, the diploid male might be considered an approach toward femaleness on the basis of antennal segment number. This erroneous suggestion, frequently made, originates in the older, incorrect concept that diploidism in itself causes femaleness in Habrobracon. Because of chromosome number, differences between diploid and haploid males are to be expected. However, these differences are not in the direction of femaleness. Cell size is a striking example of this. Since haploid males have cell size approaching that of diploid females, cells unusually large for their chromosome number are characteristic of maleness. When cell size in diploid males considerably surpasses that of diploid females the tendency may be considered as increased maleness.

Likeness, seeming intermediacy in antennal segment number should not be regarded as intersexuality in diploid males. Because of increased cell size diploid male antennal segments tend to be longer. Under the limit in structural size, which seems imposed on polyploid animals, diploid male antennae have to be composed of fewer of these longer segments. Thus, difference in antennal segment number between diploid and haploid males is at least indirectly traceable to chromosome number while true sex differences are due to heterozygosity of the sex factor. This explanation throws light on a similar situation observed with the sex-linked gene "fused" (P. W. Whiting, 1943). Here, mutation causes reduction in number and fusing together of antennal segments (P. W. Whiting, 1935) in such a way that differences due to sex tend to be masked, although differences due to chromosome number are conspicuous.

Even slight tendency toward intersexuality such as shorter male and

longer female antennae is not common. The material of the present investigation is derived from six of the stocks maintained specifically for sex allele studies. None of the classes of offspring showed such intersexual tendency in antennal length. Stability of structural secondary sex characters is surprising. Even in individuals where two types of male tissue interact to form feminized abdomens and genitalia there is no shortening of antennae suggesting feminization (Whiting, Greb, and Speicher, 1943).

Summary

1. Three types of crosses of Habrobracon, each involving only two sex alleles, were selected for investigation of diploid male mortality. The offspring gave high, low, and intermediate ratios of diploid males to females respectively, indicating low, high, and intermediate diploid male mortality. The diploid male low ratio was very low (.0167).

2. A tendency toward larger cell size in diploid male material with higher mortality is indicated in microchaetae evidence. The tendency is not extreme in the adult animals studied presumably because extreme types did not survive to the adult stage. Evidence of selective action of mortality is shown by differences between frequency distribution curves of diploid males and the curves of more viable types.

3. In all material investigated diploid male cell size was significantly larger than female cell size. Haploid male cell size approached female cell size. This is shown by number of microchaetae per unit area of wing surface and by measurements of eye facets. To explain diploid male mortality it is suggested that doubled gene situations in the diploid male, homozygous for a sex allele, might function poorly under the surface-volume relationship of the large diploid male cell. The large size of diploid male cells may itself be the result of gene duplication. The system which raises haploid male cells to a size comparable with diploid female cells may be increased in activity when its determiners are doubled in the homozygous sex allele condition of the diploid male.

4. No statistically significant differences are shown in body length between diploid males and females. These data give a statistical basis for general observations that diploid males do not exhibit gigantism. Haploid male mean body length is shown consistently smaller and

illustrates the tendency toward dwarfism of the haploid male. Wing length data supplement those of body length.

5. Larger eyes of the male are a secondary sex character not affected by cell size differences. This sex character is not as clear cut as antennal length.

6. Long antennae of the male are a secondary sex character. In none of the material was there significant shortening of antennae which would be considered tendency toward intersexuality. Such stability of secondary sex character is discussed on the basis of the sex allele being a differential chromosome segment. Female antennae are shorter chiefly because of fewer segments.

7. Diploid males have a tendency toward fewer and longer antennal segments than haploid males. Fewer antennal segments in the diploid male are not regarded as due to intersexuality but as an indirect effect of chromosome number.

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THE EFFECTS OF THIOUREA* ON AMPHIBIAN DEVELOPMENT[1]

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A. INTRODUCTION

It is well known that modification of thyroid size and activity can be induced by the administration of certain diets or special chemical substances. In particular, leaves of cabbage and other members of the Brassica family have been found to produce thyroid hyperplasia when fed to mammals (Chesney, Clawson, and Webster, '28; Webster, Clawson, and Chesney, '28; Webster and Chesney, '28; Marine, Baumann, and Cipra, '29; McCarrison, Sankaran, and Madhara, '33; and Spence, Walker, and Scowen, '33) or amphibia (Borland, '43). Thiocyanates (Geimenhardt, '38) and nitriles (James, '39) have been believed to be responsible for these effects in cabbage. Marine and his associates ('32) were able to reproduce the goiterogenic effect by injection of cyanides. Soya beans which are rich in cyanogens are also effective goiterogenic agents (McCarrison, '33; Sharpless, Pearsons, and Prato, '39).

The generally accepted interpretation of these effects is that such compounds by depressing cellular metabolism create a greater demand for active thyroid principle which, in turn, increases the need of the gland for iodine. The thyroid attempts to compensate by becoming enlarged. In support of this explanation is the finding that the administration of inorganic iodine will prevent the occurrence of this goiterous state (Chesney, Clawson, and Webster, '28; Webster, '29).

Overgrowth of the thyroid glands of rats has also been produced by the seeds of all Brassica species including Swede, soft and hard turnip, and rape (Hercus and Purves, '36; Kennedy and Purves, '41). Kennedy ('42) in an attempt to isolate the goiterogenic agent from

*With a note on the ineffectiveness of sulfadiazine and para-aminobenzoic acid.

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rape seed was led to suspect that a thiourea compound was responsible for the effect. He demonstrated, in addition, that allylthiourea induced marked hyperplasia of the rat thyroid. The Mackenzies and McCollum ('41) were the first to report such activity for the sulfonamides. The findings with thiourea and thiouracil have been confirmed and extended for the mammal by Richter and Clisby ('41 and '42), the Mackenzies ('43), Astwood *et al* ('43), Baumann, Metzger, and Marine ('44) and for the chick, by Mixner, Reineke, and Turner ('44) and Astwood, Bissell, and Hughes ('44). A state of functional hypothyroidism is always associated with the thyroid hyperplasia induced by thiourea compounds. The goiterogenic effects of Brassica seeds or thiourea, related substances and the sulfonamides cannot be overcome by iodine but may be readily prevented by administration of thyroid substance or by removal of the hypophysis (Griesbach, Kennedy, and Purves, '41; the Mackenzies, '43; and Astwood *et al*, '43).

The developing amphibian lends itself well to a study of agents which influence thyroid activity. Such effects are reflected sensitively in the rate of hind limb growth, time of forelimb extrusion and loss in body weight. For this reason and with the hope that additional information concerning the mode of thiourea action would be secured, a detailed study was made of the effects of this drug on amphibian development.

A preliminary report indicating that thiourea inhibits metamorphosis in the amphibian has already been published (Gordon, Goldsmith, and Charipper, '43). Similar effects on the amphibian with thiouracil have been noted by Hughes and Astwood ('44). A comprehensive list of compounds sharing the goiterogenic properties of thiourea appears in the study of Astwood ('43a).

B. Materials and Methods

Rana pipiens larvae were obtained in large numbers by the experimental ovulation method described by Rugh ('34). They were reared in tap water which was changed daily and kept at room temperature (23-26° C.). Crowding factors were precluded by placing relatively small numbers of animals in pans containing large amounts of water. Except where specified all animals were maintained exclusively on a diet composed of ample quantities of boiled spinach.

In the first series of experiments, 200 tadpoles were allowed to develop normally until they attained a total body length of 20-30 mm. Half of these continued their development in tap water. The remaining animals were immersed in tap water containing 0.33 per cent thiourea (Eimer and Amend), changed daily. This concentration was found to be toxic and was reduced gradually over the course of two weeks to 0.033 per cent. Measurements of total length, body length (from tip of snout to base of tail), and hind limb length were taken after 50 and 88 days of immersion. At the time of each measurement the animals were placed in nets, the excess water allowed to drain for about a minute, and then they were weighed to the nearest tenth of a gram on a trip balance. A study of the skeletal system development of the thiourea-treated animals was made by means of X-rays. The radiographs were compared with those made from control animals during different stages of development. Before exposure to the X-rays, all animals were anesthetized in a saturated solution of chlorobutanol. A non-screen film was employed for all exposures; the distance from the anode to the object was 36 inches. All films were exposed for 0.5 seconds; 30 milliamperes of current was employed. The wave lengths varied according to the thickness of the animal; 30-65 kilovolts were employed.

In a second experiment, 68 tadpoles which had progressed to approximately the 7.0 and 13.0 mm. hind limb stages were divided into two groups. Several of the animals were allowed to continue untreated, and the others were immersed in 0.033 per cent thiourea solutions.

In a third experiment, two groups of 12 tadpoles which had been exposed to 0.033 per cent thiourea solutions for 42 and 112 days were returned to ordinary tap water and measurements taken at frequent intervals.

In a fourth experiment, 170 tadpoles which had attained a hind limb length of approximately 4.0 mm. were divided into two groups. Half of these received a preliminary treatment consisting of five pleuroperitoneal injections of 1 mg. of thiourea in 0.05 cc. of distilled water over a period of 10 days. On the eleventh day and every second day thereafter they were given 0.05 cc. injections of 1-200 thyrotrophic hormone;[2] they continued to receive 1 mg. injections

[2]We wish to thank Dr. Oliver Kamm, of Parke, Davis and Company, for a supply of Antuitrin-T.

of thiourea on the alternate days. The 85 controls which had been given five preliminary injections of 0.05 cc. distilled water over a 10-day period were then injected with 1-200 thyrotrophic hormone once every two days; on the alternate days, 0.05 cc. injections of distilled water were administered. No food was given to either group of animals during the experimental period.

In a fifth experiment, 36 animals which had been immersed in 0.033 per cent thiourea for 63 days were employed. Twelve were then injected with thyrotrophic hormone. The hormone dosage was started at a level of 1-200 and increased gradually to 1-10. These animals remained in 0.033 per cent thiourea solutions throughout the experiment. Twelve animals in a second group were immersed in daily renewed solutions containing 1-100 million thyroxine (Hoffman-LaRoche) and 0.033 per cent thiourea. The 12 animals in the third group received only the thiourea immersion treatment. No food was given during the experimental period.

In the final experiment, 135 tadpoles which had attained approximately the 5.0 mm. hind limb stage were separated into three groups. All animals in these groups received injections of 1-200 thyrotrophic hormone once every two days. On alternate days the animals of one group were injected with 2 mg. sulfadiazine,[3] those of the second group with 1 mg. para-aminobenzoic acid (brought to a pH of 6.0) and those of the third group with distilled water. The sulfadiazine animals had received two injections and the PABA animals, six injections prior to the initiation of the thyrotrophic hormone treatment. The animals received no food during the experimental period.

At least three representative animals from all experimental and control groups were decapitated after various periods of treatment and the heads prepared for histological examination of the thyroid glands. Bouin's and Zenker-formol fixatives were employed and the staining was carried out with either hematoxylin-eosin or Masson's.

C. Results

1. *General Observations on Young Tadpoles (20-30 mm.—Initial Total Length)*

An examination of Table 1 indicates clearly that thiourea inhibits

[3]We are indebted to Dr. John Henderson, of Sharp and Dohme Incorporated, for a generous sample of sulfadiazine.

TABLE 1

EFFECTS OF THIOUREA ON DEVELOPMENT OF YOUNG RANA PIPIENS LARVAE

	EXPERIMENTALS (Thiourea)					CONTROLS (Tap water)				
Days of experiment	No. of animals	T.L. (mm.)	B.L. (mm.)	H.L. (mm.)	Wt. per animal (gm.)	No. of animals	T.L. (mm.)	B.L. (mm.)	H.L. (mm.)	Wt. per animal (gm.)
0	100	20-30	——	—	—	100	20-30	——	—	—
50	70	51.9	19.0	2.3	3.2	98	48.6	17.7	5.6	1.5
88	45	80.3	31.0	4.8	4.9	95	90% forelimbs			2.1

T.L., B.L., and H.L. refer respectively to the mean total, body, and hind limb lengths.

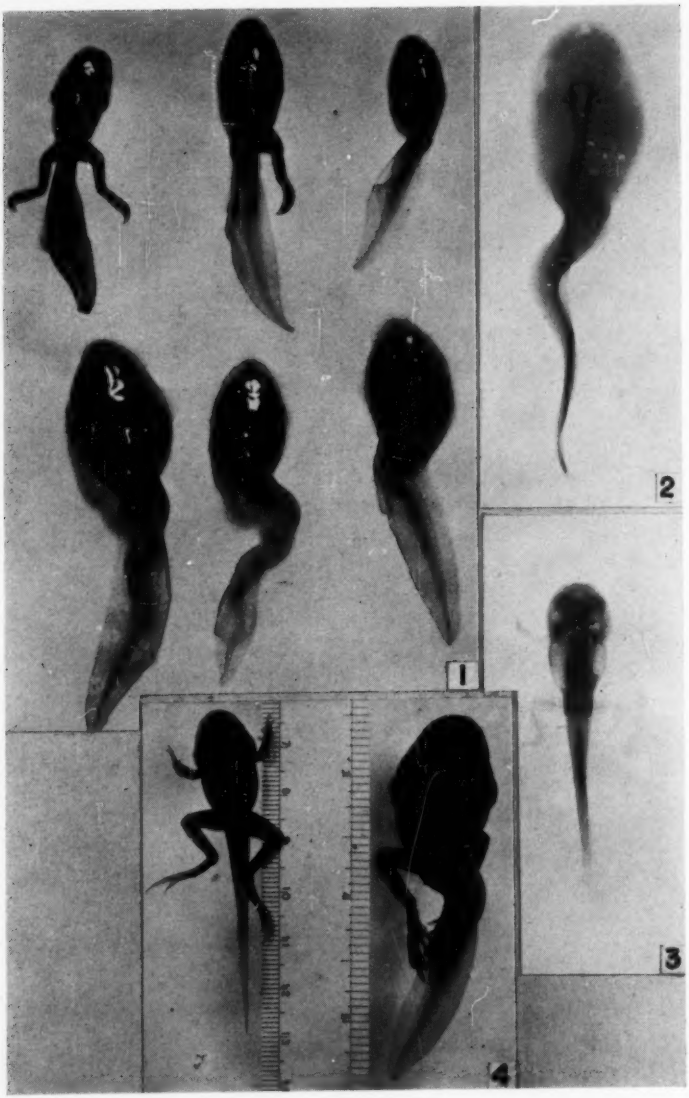
1
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PLATE I

(Figures 1-4)

1. Note the larger size, arrested development and crooked tail base condition in the tadpoles immersed in 0.033 per cent thiourea for 58 days since the 20-30 mm. total length stage (lower animals) as compared with normal Rana pipiens larvae in different stages of development (top animals).

2. X-ray photograph of a tadpole immersed in 0.033 per cent thiourea for 58 days since the 20 mm. total length stage. Note the limited degree of ossification in the axial skeleton.

3. X-ray photograph of a normal tadpole of the same chronological age as the one shown in Figure 2. Note the greater extent of ossification of the axial skeleton.

4. Recently metamorphosed Rana pipiens larvae. Animal on the left is a normal frog (chronological age—104 days). Animal on the right had been in 0.033 per cent thiourea for 112 days since the 25 mm. total length stage and then returned to tap water for 102 additional days (chronological age—232 days). Note the much larger overall dimensions of the frog on the right.

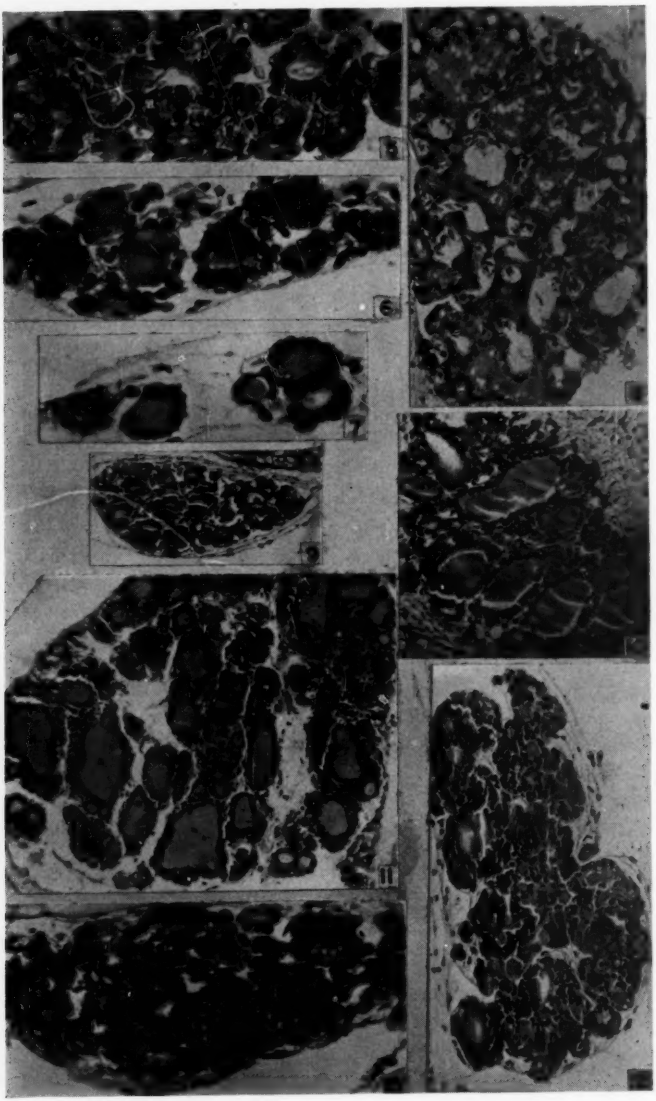

PLATE II

(Figures 5-13)

5. Thyroid of young larva immersed in 0.033 per cent thiourea for 21 days since the 25 mm. total length stage. Note the marked hyperplasia, collapsed follicles, and scanty vacuolated colloid. Bouin; hematoxylin-eosin. X 220.

6. Thyroid of untreated control larva of the same chronological age of the experimental whose gland is shown in Figure 5. Gland is by no means as active or as enlarged as the experimental. Bouin; hematoxylin-eosin. X 220.

7. Thyroid of larva immersed in 0.033 per cent thiourea for 86 days since the 25 mm. total length stage. Marked regression in size and activity of the gland is noted when compared with the thyroid shown in Figure 5. Bouin; hematoxylin-eosin. X 220.

8. Thyroid of larva immersed in 0.033 per cent thiourea for 18 days since the 7.0 mm. hind limb stage. Note the goiterous proportions and marked histological activity of the gland. Bouin; hematoxylin-eosin. X 88.

9. Thyroid of untreated control larva of the same chronological age as the experimental shown in Figure 11. Although this gland is very much smaller than that in the experimental, the extent of its histological activity is only slightly less. Bouin; hematoxylin-eosin. X 66.

10. Thyroid of untreated control larva of the same chronological age as the experimental whose gland is shown in Figure 8. Gland is considerably smaller and less active than the experimental one. Bouin; hematoxylin-eosin. X 88.

11. Thyroid of larva immersed in 0.033 per cent thiourea for 40 days since the 7.0 mm. hind limb stage. Note the marked goiterous proportions and numerous follicles but signs of regression in histological activity are now noticeable (compare with Figure 8). Bouin; hematoxylin-eosin. X 66.

12. Thyroid of larva immersed in 0.033 per cent thiourea for 86 days since the 25 mm. total length stage and then returned to tap water for 42 additional days. Gland has been restored to a histologically active condition (compare with Figure 7). Bouin; hematoxylin-eosin. X 88.

13. Thyroid of larva immersed in 0.033 per cent thiourea for 63 days since the 25 mm. total length stage and then given five injections of thyrotrophic hormone over a 10-day period in addition to the thiourea treatment. The inactive histological condition has been converted to an extremely active one. Zenker-formol; Masson. X 176.

the advance of the metamorphic process in the young tadpole. Very little progress is made in development even after 88 days, a time when most of the controls had completed their metamorphosis. Such animals, however, continue to grow and attain weights and proportions much larger than those displayed by any of the controls (Figure 1). The body and head, in particular, show considerable broadening and the viscera are unusually large. X-ray studies made of the treated animals reveal a significant inhibition in skeletal differentiation. Although the skeleton, as a whole, continues to grow and attains dimensions in proportion to the size of the animal, ossification processes are significantly retarded (cf. Figures 2 and 3). The degree of development of the skeletal system in the animals treated with thiourea for 88 days compares favorably with that present in the considerably younger untreated controls in approximately the same state of metamorphic development (i.e., 4-8 mm. hind limb stage).

Following about a month of treatment most of the animals develop peculiar tail abnormalities characterized by a twisting confined to the basal region (Figure 1). Radiographic and histological studies of these tails reveal no abnormal skeletal growth but rather an overgrowth of the connective tissue in the distorted region. This phenomenon is observed only rarely in the untreated controls.

2. *Thyroid Histology in Young Tadpoles (20-30 mm.—Initial Total Length)*

For about the first week of thiourea treatment no striking changes are noticeable in the morphology of the thyroid gland. By the tenth day a slight enlargement is observed. The gland at this time is markedly hyperemic. The height of the follicular epithelium is, however, still unaltered, being of the flattened or cuboidal type in both the experimental animals and the untreated controls; considerable non-vacuolated colloid is also visible in both. After 14 days of immersion, the gland is again slightly larger than that of the control. The individual thyroid follicles of the experimental animals are of approximately the same size, but are more numerous and contain less colloid than in the untreated larvae. The epithelium remains cuboidal.

By the third week of treatment, the gland is highly active (cf. Figures 5 and 6). The individual follicles are larger and more numerous. They display irregular shapes and infoldings due probably

to crowding resulting from the marked overgrowth; the epithelium is predominantly columnar. Mitotic figures among the follicular cells are numerous. Small amounts of highly vacuolated colloid remain in the lumina of some follicles of these hyperplastic glands; other follicles are completely collapsed. Vascularity is markedly increased and the individual vessels are engorged with blood elements. In many cases the blood vessels tend to obscure the cellular elements of the gland. This intensely active picture of the thyroid gland is maintained up to approximately 50 days of treatment. The glands in animals of this series tend to be somewhat larger, as a whole, than those of untreated larvae but never attain goiterous proportions. The presence of collapsed follicles, columnar epithelium and marked hyperemia are almost invariable features of the gland after three to seven weeks of thiourea immersion.

A regression in the histological activity of the thyroid is apparent after approximately two months of treatment. The follicles assume smaller size and the follicular epithelium returns to the cuboidal condition. Colloid of a fairly dense nature accumulates in the lumina. The gland, as a whole, is decreased in size. The hyperemic condition, however, still persists. After 75 days of treatment, the gland is still smaller in size. Densely staining and occasionally streaked colloid distends these follicles. The epithelium is flattened; in a few cases, it remains cuboidal. By the ninetieth day, the gland is small and, in some cases (Figure 7), atrophic. A median section through the gland contains no more than two to five follicles. These follicles are small and possess either a cuboidal or flattened epithelium. A high degree of hyperemia, however, is still apparent. This inactive thyroid picture is in sharp contrast to the considerably larger and highly active glands in metamorphosing controls of the same chronological age.

3. *Observations in Older Tadpoles*

Tadpoles immersed in 0.033 per cent thiourea solutions at a later stage in development (i.e., 7.0 mm. hind limb length) continue to advance in metamorphosis but at a less rapid rate when compared with untreated controls, as shown in Table 2. Whereas all control larvae had metamorphosed after 15 days, not one of the experimental had extruded forelimbs.

TABLE 2

EFFECTS OF THIOUREA ON DEVELOPMENT OF OLDER RANA PIPIENS LARVAE

Days in thiourea	EXPERIMENTALS H.L. (mm.)	CONTROLS H.L. (mm.)
0	7.0(12)	7.1(12)
8	16.1(12)	18.2(12)
11	20.7(10)	25.1(8) 4 F L
15	23.7(7)	30.8(8) 8 F L
18	30.0(6)	———
30	33.1(6)	———

H.L. refers to the mean hind limb length; F L to the number reaching the forelimb stage. In parentheses are indicated the numbers of animals.

Enlargement of the thyroid glands of animals in this group is much more striking than that occurring in younger tadpoles (i.e., 20-30 mm.—initial total length). This overgrowth is first observed after about eight days of immersion, a time when the animals have attained the 16 mm. hind limb stage (Table 2). The size of the gland is still greater after 11 days. Following 18 days of treatment the gland has attained goiterous proportions (cf. Figures 8 and 10). There is a striking increase in the numbers of follicles. Many of these are irregularly shaped and display tortuous infoldings which tend to obliterate the lumina. These ingrowths appear to be forming new follicles by a process of budding. In some cases, cord-like masses of epithelial cells without visible lumina appear to have proliferated among the hyperplastic follicles. No signs of necrosis are ever noticeable in such glands. A good number of the follicles are collapsed and the remaining scanty colloid is highly vacuolated at the periphery. The acinar cells are columnar and the cytoplasm assumes an emulsion-like appearance. The enlarged glands are invariably hyperemic. The thyroids from 30-40 day-thiourea-immersed animals are even larger in size. Some of these, however, no longer reveal the intense activity characteristic of glands from the 11- or 18-day treated animals (cf. Figures 11 and 9). A few, however, remain histologically active for as long as three months of treatment. A definite decrease in activity of the glands is noted after approximately four months of treatment. This degree of regression, however, is never as striking as that observed in younger larvae treated for the same period of time.

In another series of experiments not included in the tables, 22 larvae which had been permitted to progress normally to the 13.0

mm. hind limb stage were then immersed in 0.033 per cent thiourea solutions. Although such treatment did not prevent the extrusion of the forelimb, definite retardation of the tail resorption process was observed.

4. *Reversibility of the Thiourea Effect*

That the effects of thiourea on development are reversible can be seen from the data in Tables 3 and 4. If the animals are exposed to thiourea for 42 days and then returned to tap water, metamorphosis is not only promptly resumed but is actually completed in a shorter time than that taken by the untreated larvae. These animals continue to increase in size until the time of forelimb emergence (Figure 4). Immersion for longer periods of time (112 days), however, appears to exert some injurious effect since the rate of resumed development in tap water is considerably delayed. The extremely large dimensions and weights attained by the animals treated with thiourea are to be

TABLE 3

REVERSIBILITY OF THE THIOUREA EFFECT

Days out of thiourea	T.L. (mm.)	B.L. (mm.)	H.L. (mm.)
0	66.5	24.7	3.5
4	70.4	26.1	4.2
8	75.0	28.0	7.0
13	77.0	27.0	15.8
20	83.3	27.3	28.3 (2 F L)
24	84.6	28.5	35.0 (4 F L)

T.L., B.L., and H.L. refer respectively to the mean total, body and hind limb lengths of six animals exposed to thiourea for 42 days and then returned to tap water. In parentheses are indicated the numbers of animals attaining the forelimb emergence (F L) stage.

TABLE 4

REVERSIBILITY OF THE THIOUREA EFFECT

Days out of thiourea	T.L. (mm.)	B.L. (mm.)	H.L. (mm.)	Wt. per animal (gm.)
0	86.0	33.6	5.6	5.9
7	87.0	33.6	6.0	6.3
12	86.3	33.8	6.5	7.2
18	86.8	34.8	7.3	7.4
23	90.0	35.3	8.5	8.0
28	90.0	34.8	11.3	7.9
44	88.0	34.0	15.7	8.1

T.L., B.L., and H.L. refer respectively to the mean total, body and hind limb lengths of six animals exposed to thiourea for 112 days and then returned to tap water.

TABLE 5

EFFECTS OF THYROTROPHIC HORMONE OR THYROXINE ON LONG-TIME THIOUREA-IMMERSED ANIMALS

THYROTROPHIC HORMONE				THYROXINE IMMERSED				THIOUREA ONLY			
No. of injections	T.L. (mm.)	B.L. (mm.)	H.L. (mm.)	No. days in thyroxine	T.L. (mm.)	B.L. (mm.)	H.L. (mm.)	No. addit. days in thiourea	T.L. (mm.)	B.L. (mm.)	H.L. (mm.)
0	61.4	21.5	3.0	0	62.5	21.9	3.4	0	62.0	22.1	3.1
2	62.0	22.3	3.0	4	61.7	22.1	7.0	4	64.5	23.3	3.3
5	60.1	21.5	3.0	8	54.4	19.4	16.2(6 F L)	8	61.6	22.6	3.1
7	60.5	21.8	3.0	10	54.3	20.3	(All F L)	16	58.3	21.6	3.2
8	58.4	20.2	3.0	—	—	—	—	—	—	—	—

T.L., B.L., and H.L. refer respectively to the mean total, body and hind limb lengths of three groups of 12 larvae immersed in thiourea for 63 days. In parentheses are indicated the numbers of animals attaining the forelimb emergence (F L) stage.

seen especially in this group. The ability of the larvae immersed in thiourea for a long time to renew their differentiation when returned to tap water is due undoubtedly to the restoration of the thyroid gland in these animals to a functional condition (Figure 12). The atrophic picture in this gland is changed to a normal one as early, in some cases, as the first week after discontinuation of thiourea treatment.

5. *Effects of Thyrotrophin on Larvae Treated with Thiourea*

Thiourea is also capable of antagonizing the effects of thyrotrophic hormone on tadpole development. Immersion in thiourea solutions for about two months will completely inhibit the effects of subsequently injected thyrotrophic hormone on development as shown in Table 5. The thyroid glands of these tadpoles injected with thyrotrophin, however, are enlarged and show signs of considerable activation (Figure 13). Control animals immersed in thiourea for the same period of time and given no thyrotrophin possess atrophic glands. If injections of thiourea are given instead of immersions, the animals require to be primed for at least a week with the drug before the hormone is administered (Table 6). Even under these conditions some advancement does occur but this does not compare with the marked acceleration of development taking place in the animals injected with only thyrotrophic hormone.

If thiourea immersion is continued for too long a period of time (e.g., 110 days), the ability of the thyroid to respond to thyrotrophin injections is reduced. In some cases, peculiar large cyst-like structures are produced in response to injections of the hormone.

TABLE 6

EFFECT OF THIOUREA ON THYROTROPHIC HORMONE

No. of thiourea inject.	No. of thyrot. inject.	H.L. (mm.)	No. of thyrot. inject.	H.L. (mm.)
0	0	4.4	0	4.4
7	2	5.5	2	6.9 (3 F L)
8	4	6.0	4	7.2 (45 F L)
9	5	6.4	—	
12	8	6.8 (1 F L)	—	

H.L. refers to the mean hind limb lengths of two groups of 85 larvae. In parentheses are indicated the numbers of animals attaining the forelimb emergence (F L) stage.

6. *Effects of Thyroxine on Larvae Treated with Thiourea*

Animals which have been arrested in their development with thiourea can be made to undergo metamorphosis at any time by the addition of small quantities of thyroxine to the thiourea solutions (Table 5). Fully formed frogs emerge after about two weeks of treatment with dilutions of thyroxine as high as 1 part in 100 million parts of water.

7. *Non-Effectiveness of Sulfadiazine and Para-Aminobenzoic Acid*

Table 7 shows that neither sulfadiazine nor para-aminobenzoic acid is capable of inhibiting the effects of thyrotrophic hormone on the development of Rana pipiens larvae.

TABLE 7

EFFECT OF SULFADIAZINE AND PARA-AMINOBENZOIC ACID (PABA) ON THYROTHOPHIC HORMONE

SULFADIAZINE			PABA			CONTROLS	
No. thyrot. inject.	No. sulfad. inject.	H.L. (mm.)	No. thyrot. inject	No. PABA inject.	H.L. (mm.)	No. thyrot. inject	H.L. (mm.)
0	0	5.5	0	0	5.4	0	5.0
3	5	10.0	3	9	8.2	3	8.0
4	6	(33 F L)	4	10	(40 F L)	4	(35 F L)

H.L. refers to the mean hind limb lengths of three groups of 45 larvae. In parentheses are indicated the numbers of animals attaining the forelimb emergence (F L) stage.

D. DISCUSSION

In recent reports the Mackenzies ('43) and Astwood *et al* ('43) have described striking effects on thyroid gland activity following the administration to rats of thiourea, its derivatives, and certain of the sulfonamides. These drugs induce an enlargement of the thyroid associated with a state of functional hypothyroidism. The results of the present investigation point to a similar condition occurring in amphibia treated with thiourea. Frog larvae subjected to the action of the drug at an early stage in development (initial total length 20-30 mm.) make practically no progress in metamorphosis. Growth, however, is not inhibited but, after a time, actually becomes excessive. The large dimensions and weights attained by the animals are striking. Such results indicate strongly that thyroid function is impaired by thiourea treatment. The tadpoles, in fact, resemble closely the com-

pletely thyroidectomized larvae described by Allen ('18) and Hoskins and Hoskins ('19). The effects on the skeleton are quite similar to those reported by Terry ('18) in the thyroidless tadpole.

Despite the fact that these animals fail to show any signs of advanced differentiation, the histological picture of the thyroid after a few weeks of treatment is one of intense activity. The enlarged hyperemic and hyperplastic glands are reminiscent of those found in higher animals suffering from hyperplastic or toxic goiter. The paradoxical situation of thyroid hyperplasia occurring coincidentally with a state of functional hypothyroidism in animals treated with thiourea appears to have the following explanation. Studies with radioactive iodine have shown that thiourea (Keston *et al,* '44) or thiouracil (Rawson, Tannheimer, and Peacock, '44; Franklin, Lerner, and Chaikoff, '44) interferes with the conversion of inorganic to organic iodine. It has also been suggested (Astwood, '43b; Dempsey and Astwood, '43) that such compounds prevent the transformation of diiodotyrosine to thyroxine. The anterior lobe of the pituitary responds to this lowered thyroxine content of the blood by releasing more thyrotrophic hormone which soon induces a hyperplastic enlargement of the thyroid gland. That the pituitary is concerned with the response has already been indicated (the Mackenzies, '43; Astwood *et al,* '43) since hypophysectomy prevents the thyroid hyperplasia. Moreover, thiourea and related compounds cause the appearance of "thyroidectomy cells" in the anterior lobe of the pituitary. This mechanism appears to adequately explain the results obtained in the mammal and, most likely, in the amphibian. The hypothesis receives additional support from the findings that thyroxine, but not iodine, will restore the basal metabolism to normal levels in rats fed thiourea (the Mackenzies, '43; Astwood *et al,* '43) and prevent the thyroid hyperplasia in treated rats and chicks (the Mackenzies, '43; Astwood *et al,* '43; Mixner, Reineke, and Turner, '44).

Although the hyperplastic condition of the thyroid in the rat treated with thiourea or the sulfonamides can be maintained for periods as long as six to eight months (Astwood *et al,* '43; the Mackenzies, '44; unpublished observations), this is not the case for the amphibian. In the tadpoles subjected to thiourea early in development, the gland, after a period of intense activity, becomes reduced in size following approximately three months of treatment, and in the older larvae,

after about four months. This regression in size and histological activity of the gland might be attributed either to a refractoriness developed by the thyroid to endogenously produced thyrotrophic hormone or to a failure in the thyrotrophic hormone production and/or release mechanism of the pituitary. The first possibility is precluded and the second strongly supported by our observation that thyrotrophic hormone injections will transform the hypoplastic condition in the thyroid of animals treated with thiourea for a long time to one of high activity. The eventual injury to the hypophysis appears to be more pronounced in the younger than in the older tadpole. The results also indicate that the thyroid itself may eventually become injured as a result of protracted treatment with thiourea. This is shown by our finding that the tadpole gland fails to respond normally to thyrotrophin injections if thiourea immersions are given for 110 days. Williams and Bissell ('43) and more recently McGavack *et al* ('44) have reported a regression in the size of the thyroid in some of their hyperthyroid patients treated with thiouracil. This might be interpreted to mean that the drug has produced a partial inhibition of the pituitary thyrotrophic hormone production and release mechanism or has diminished the receptivity of the thyroid to endogenously-produced thyrotrophin in these individuals. The question of eventual pituitary failure and thyroid injury in animals under continuous treatment with thiourea or its derivatives is one of importance both from a fundamental and clinical point of view, and warrants further examination.

The data indicate that older tadpoles (7.0 mm. hind limb length) immersed in thiourea solutions continue to advance in metamorphosis but at a significantly slower rate than that exhibited by the untreated controls. This continuation in development is due probably to the release of thyroid hormone stored in the gland before initiation of the thiourea treatment. Release of preformed thyroxine from the thyroid for a short time following thiouracil treatment has also been reported in the rat (Dempsey and Astwood, '43) and in the human (Astwood, '44). Recent unpublished observations indicate very little further advance in more mature treated larvae (initial hind limb length —7.0 mm.) after 30 days. Forelimbs are not extruded even after two to three months in animals of this series.

The X-ray studies reveal that thiourea inhibits the process of ossi-

fication in the tadpole. A state of cretinism has also been induced in rats under the influence of thiouracil (Hughes, '44) or thiourea (Goldsmith, Gordon, and Charipper, in press). The possibility exists that the tail abnormality occurring in the treated larvae represent a peculiar manifestation of cretinism in the tadpole. The X-ray and histological studies show, however, that this condition cannot be attributed to any deformity in the skeleton at this region but rather to an extensive overgrowth and folding of the connective tissue. Moreover it must have been due to the thiourea itself rather than to any peculiar environmental condition such as the water (Allen, '18) or the food (Hoskins and Hoskins, '19) since it develops only rarely in the untreated controls.

One of the most interesting features of thiourea action is the reversibility of its effect. It has been indicated in this report that the development of frog larvae can be kept in complete abeyance for as long as thiourea treatment is administered (e.g., 112 days). Signs of resumed metamorphosis, however, are displayed very early following discontinuation of treatment, provided the immersion is not continued for too long a time. Similarly, in the rat, Astwood *et al* ('43) have reported a regression in thyroid size following the withdrawal of thiourea. It is also known that growth can be arrested in rats by feeding thiouracil (Hughes, '44; Williams *et al*, '44) or thiourea (Gordon, Goldsmith, and Charipper, in preparation). Certain of our own experiments have shown that the removal of thiourea or thiouracil from the diets of young or adult rats will result in a rapid resumption of the normal growth rate. Dempsey ('44) has postulated that peroxidase catalyzes the formation of thyroxine and has demonstrated that thiouracil inhibits this enzyme in thyroid follicular cells. Thiourea also interferes with peroxidase activity (Sumner and Somers, '43). If this is the case then it must also be true that the enzyme is quite readily replaced or restored to activity upon withdrawal of the drug. If, however, immersion of the tadpoles in thiourea solutions is continued for too long a time (i.e., 112 days) resumption of development in tap water may be considerably delayed. In this connection, Astwood ('44) has recently reported that the treatment of hyperthyroid patients with thiouracil for relatively long periods of time may be attended by continued remission of the toxic symptoms even after withdrawal of the drug.

It was surprising to find that sulfadiazine, even in relatively large amounts, is unable to inhibit thyroid action in the tadpole in view of its marked activity in the mammal (the Mackenzies, '43; Astwood *et al,* '43). This probably represents a difference in the response shown by the amphibian and mammalian thyroid to the sulfonamides. A similar species difference has been observed in the chick, in which animal the feeding of sulfaguanidine (the Mackenzies, '43) or sulfadiazine (Astwood, Bissell, and Hughes, '44) is incapable of evoking thyroid enlargement. Recent reports (Mixner, Reineke, and Turner, '44; Astwood, Bissell, and Hughes, '44), however, have shown that thiouracil can induce thyroid overgrowth in the chick. The Mackenzies have suggested (personal communication) that the variation in response might be attributed possibly to a difference in the manner by which the sulfonamides are utilized and metabolized by the different species.

In every species of animal thus far examined, however, thiourea and its derivatives have been found to be effective agents in reducing physiological activity of the thyroid. The animals already tested include the rat (the Mackenzies, '43; Astwood *et al,* '43), mouse and dog and (the Mackenzies, '43), rabbit (Baumann, Metzger, and Marine, '44), human (Astwood, '43b; Williams and Bissell, '43; Himsworth, '43; Sloan and Shorr, '44; Rawson *et al,* '44; Paschkis *et al,* '44; Astwood, '44; McGavack *et al,* '44), chick (Mixner, Reineke, and Turner, '44; Astwood, Bissell, and Hughes, '44), tadpole (Gordon, Goldsmith, and Charipper, '43; Hughes and Astwood, '43; this work), and fish (Goldsmith *et al,* '44). These findings constitute indirect but convincing evidence for the contention that a similar chemical mechanism operates for the production of thyroid hormone in at least four classes of the vertebrates.

E. Summary

1. Thiourea inhibits the process of metamorphosis in Rana pipiens larvae; growth, however, continues and becomes excessive. X-ray studies indicate a retardation in ossification processes.

2. Treatment of the young tadpole (i.e., initial total length—20-30 mm.) with thiourea for three weeks results in the development of a slightly enlarged but markedly activated thyroid gland (columnar epithelium, scanty colloid, marked hyperemia, etc.); this condition is

maintained until the seventh week. A marked regression in the histological activity of the thyroid is apparent after approximately two months of treatment; this regression is due to a failure in the thyrotrophic hormone production and/or release mechanism of the pituitary.

3. Immersion of older larvae (initial hind limb length—7.0 mm.) in thiourea solutions for two to six weeks causes a striking increase in the size and histological activity of the thyroid; moderate regression of this hyperplastic state occurs after approximately four months of treatment.

4. Larvae immersed in thiourea solutions resume metamorphosis when returned to tap water; this is accompanied by a return of the thyroid gland to normal histological condition; long periods of treatment delay this return.

5. Treatment with thiourea for a long period of time (e.g., 110 days) may cause injury to the tadpole thyroid as shown by its failure to respond normally to thyrotrophic hormone.

6. Thiourea is also capable of antagonizing the effects of thyrotrophic hormone on amphibian development; in this regard, immersion in solutions of the drug are more effective than injections.

7. The inhibitory effects of thiourea on metamorphosis can be overcome completely by thyroxine.

8. Sulfadiazine and para-aminobenzoic acid exert no action on the effects of thyrotrophic hormone in Rana pipiens larvae.

9. It is concluded that thiourea produces its effect on amphibian development in much the same way it acts in the mammal (i.e. by inhibiting the formation of normal thyroid hormone).

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EXPERIMENTAL STUDY ON THE ROLE OF TREPHOCYTES IN REGENERATION OF EISENIA FOETIDA (SAV.)*

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A. INTRODUCTION

In an earlier investigation (Liebmann, '43) regeneration and its various manifestations were found to be determined by the lymph system in general and by its main cellular components, the eleocytes, in particular.

The latter are white blood cells of a trophic character carrying nutritive and activating substances derived from the digestive system into all regions of the body and into the sexual organs and products. In cases of injury they discharge these substances into the wound.

Similar cells were observed by the writer in numerous oligochaetes and in several polychaetes and insects studied. This type of blood elements seems to be quite common in invertebrates and lower vertebrates. There exists, however, much confusion about these cells, due partly to the fact that they appear under a great number of names. It is therefore suggested to apply henceforth the term "trephocytes" to all these cells, the word indicating for the present their morphological aspect since their physiology has been but little studied.

In regeneration the trephocytes of Eisenia function as aggregations of a distinctive form. These are polarised and able to act exclusively in one direction. Unlike the individual free cells, the aggregates are function-specific and they supply only the regenerating part with the substances they carry. The clearly defined morphology and physiology thus justify considering these formations as organs: regeneration organs.

Two kinds of such organs were detected in Eisenia: a head organ

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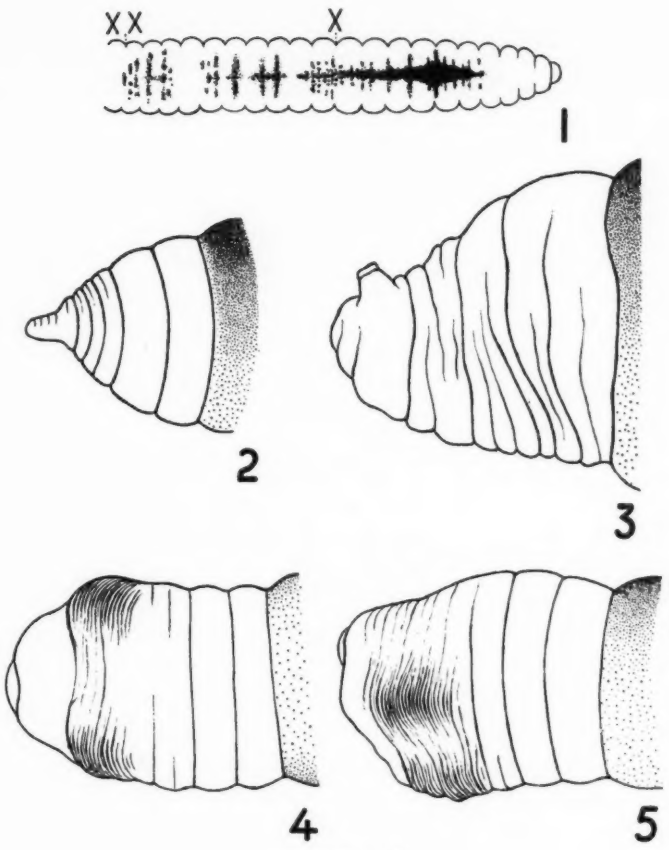
and a tail organ. The first one is of a permanent nature and instrumental in cephalic restitution; the second is formed only prior to and during the regeneration of the remainder of the body, disappearing towards the end of the process. Each of these organs exhibits gross morphological and functional traits of its own. Their respective cells show specific cyto-chemical peculiarities and are easily distinguished, both in vivo and when fixed, as head trephocytes and tail trephocytes. In the main, the tail trephocytes are characterized by lipid inclusions whilst the head trephocytes are devoid of them when in the active state.

The circumstance that head restitution is effected by a permanent regeneration organ whilst that of the tail by one of a transitory and changeable character appears to explain the diverse ways cephalic and caudal restitution respond to reproduction and nutrition. It further elucidates the cause of the mutual independence of head and tail regeneration and the independence of their respective gradients.

The rate and the amount of regeneration at a given level, were found to be proportional within limits to the number of trephocytes available for this process in the pertinent region. Hence the gradients of regeneration represent merely the resultants and expressions of similar gradients in the distribution of these cells—and ultimately the nutritive and activating substances—along the main body axis.

The fact that the trephocytes appear in two distinctive forms in the head and tail aggregates suggested that in addition to the quantitative, they may also play a qualitative rôle in regeneration. It was therefore deemed desirable to submit these questions to an experimental test by studying the effects of removal of the aggregates.

Those of the head were chosen for this purpose as, in contrast to the tail aggregates, they form permanent organs which change but little. In the adult worm the cephalic aggregates extend from segm. V posteriorly as far as segm. ± 20 (Figure 1). Their anterior portion (segms. V-XII) forms a more or less continuous mass and was termed the primary or main aggregate. The secondary aggregates occupy the segments below the twelfth and represent fragments detached from the main organ and moved posteriorly. They appear increasingly particled and irregular and their cells somewhat older than those in the primary aggregate.



FIGURES 1—5

Figure 1 is a reconstruction from serial sections and observations in vivo. Figures 2-5 are camera-lucida drawings, magnification 27x; the stippled portion represents the regenerant.

Figure 1. Location and distribution of the cephalic regeneration organ.
Figure 2. Anomalous head; case M-A 12. Lateral view.
Figure 3. Anomalous head; case M-B 12. Lateral view.
Figure 4. Anomalous head; case M-C 12. Ventral view.
Figure 5. Same head; ventro-lateral view.

B. Material and Methods

The brandling, Eisenia foetida (Sav.) was used as in previous investigations. Controls were from the same culture, of similar size and age as the individuals under experiment and were kept in identical conditions throughout it.

In order to remove the trephocytic aggregates, the head region or part of it was exposed to ether fumes, the remainder being screened off with moist filter paper. The worms respond with violent contractions and emission of lymph and trephocytes through the dorsal pores of the affected section. To ensure the elimination of a maximum possible amount, this procedure was in all cases repeated twice within a short interval in the first operation, which preceded decapitation, but in the tables it figures as a single removal. The treatment affects the worms but little as it only calls forth a natural reflex: the worms eject lymph under normal conditions also, presumably as a means of defense.

Transsections were made by a quick cut with a sharp scalpel along the intersegmental furrow. Parts of the gut which sometimes protrude above the plane of severance were trimmed off as these were observed to create a deterrent in regeneration.

The length of the regenerating heads was measured under the binocular against the nearest intact segment, both when the individuals were at rest and moving. This method proved more reliable than measurements of anesthetized worms. With the magnification applied (21X) the size was safely gauged to an accuracy of 1/5 of a segment ($= \pm 0.1$ mm.). For further study the worms were usually preserved in formalin after being killed by a short dip in hot water.

Before presenting the results, it should be stressed here that an exact experimental approach to our problem encounters several difficulties of a physiological nature, of which the more obvious are enumerated here. In the first instance, the aggregates, after being removed, are quickly reformed, particularly in the anterior head region. It was therefore necessary to repeat their elimination (usually 48 hours after decapitation), spacing the operations at such intervals so as to prevent as far as possible accumulation of these cells near the cut and their becoming active. The procedure, however, cannot be continued at will, as high mortality soon sets in, the individuals which emitted most lymph, i.e., those promising the best results, suc-

cumbing first. Last, but not least, there is the fact that the amount of trephocytes emitted varies greatly in each case and drops sharply after 4-5 operations, partly because many individuals cease to respond to ether. All this should be taken into consideration when evaluating the results.

C. Results

It is proposed to deal in the first place with the quantitative aspect of the problem, viz., with the rate of head regeneration after removal of the aggregates, and to describe subsequently its qualitative side. In both cases it is intended to proceed as far as possible in an ascending order, both in number of segments removed and operations performed.

1. *Quantitative*

a. Level of intersegment V. Forty-nine specimens were operated on with 39 surviving to the end of the operations; 23 individuals served as controls, one of them being a loss. The number of removals varied from 1-11. With one exception they were spaced at intervals of 24 hours and 12 hours. Experiments were performed by removal of the primary aggregate (I) and by emission of both (I+II). Most experiments lasted two weeks, at the end of which the regenerates exhibited well developed external features.

A summary of the results is given in Table 1. Experiments Nos. 1-5 represent a series with increasing amounts of trephocytes removed and, considering the difficulties of an exact approach, show to a high degree a corresponding decrease in rate of regeneration. This is par-

TABLE 1

No.	Number of removals	Interval between removals	Aggregates affected	7th day	14th day
1	1		I	—11.2%	—11.2%
2	4	24 h.	I	—30.6%	—11.2%
3	9	24 h.	I	—25.5%	—11.6%
4	11	24 h.	I+II	—33.8%	—10.0%
5	10	12 h.	I+II	—44.6%	*
6	7	48 h.	I	— 1.9%	—23.0%
Average:				—24.6%	—11.1%

Reduction in rate of regeneration, as compared with untreated controls; * indicates that no measurement was made on the given date but experiment was continued.

ticularly evident in the first week, when removals were continued.

As in all other cases dealt with further, regenerates in the experimental series were lagging behind, not merely in size, but also in development of segmentation and of the prostomium, to mention only the two most conspicuous external characteristics.

Experiment No. 6 did not exhibit any notable decrease in the first week, which may have been due to the far spacing of removals (each 48 hours). At the end of the emissions, however, on the 14th day, the lag in regeneration was considerable.

An analysis of the experimental series showed a greater individual variation in the rate of regeneration than in the controls. As could be expected, more individuals exhibited a very slow rate of regrowth. One specimen completely failed to regenerate for about three weeks; at this time controls showed almost fully grown regenerates.

b. Level of intersegments VIII and IX. The results of this series are given in Table 2: 38 individuals were operated on in the experimental series with 28 surviving till the end; the number of controls

TABLE 2

No.	Number of removals	Interval between removals	Aggregates affected	7th day	14th day	21st day
1	6	24 h.	I+II	—11.5%	—11.8%	*
2	6	24 h.	I+II	—41.0%	—31.5%	—10.0%
3	6	24 h.	I+II	—22.2%	0.0%	+ 2.0%
Average				—24.9%	—14.4%	— 4.0%

Explanations as for Table 1.

was 28, with 26 survivors. Experiment No. 1 was performed by amputation on intersegment VIII, the two others on inters. IX. The number of removals, their spacing and the aggregates affected (I+II) were identical in all cases. As regeneration proceeds more slowly at these levels, observations continued for 3-4 weeks. Measurements, however, were not extended beyond the 21st day as differences between the experimental series and the controls gradually disappeared towards the end of regeneration; this was observed at all levels.

Again, there was a tendency amongst the experimental worms towards an excessively slow growth: 11 individuals showed no regeneration on the 7th day against 5 in the controls. One operated worm

did not regenerate for two weeks; all controls showed regenerates at this time.

C. Level of intersegment XII. Removals of trephocytes at this level had to be limited to a maximum of three owing to high mortality. The amount of trephocytes emitted after the first operation was in most cases very small or nil, which suggested that the reformation of the aggregates in the lower levels is slow or fails completely.

Forty individuals were used for experiments with 23 survivals; 31 served as controls, 26 surviving till the end. Owing to the further decrease in rate of regeneration at this level, observations continued for 36-40 days; measurements were suspended on the 28th day.

Results are summarized in Table 3. In the experimental series the number of individuals failing to regenerate in the first three weeks was 12, 5, 2 against 7, 1, 0 in the controls. One operated specimen did not regenerate till the end of the experiment (35 days).

TABLE 3

No.	Number of removals	Interval between removals	7th day	14th day	21st day	28th day
1	3	24 h.	—66.6%	—59.0%	—26.3%	*
2	3	24 h.	—36.9%	—22.7%	+ 1.3%	—6.9%
3	2	72 h.	—21.8%	—17.4%	—12.8%	—7.7%
Average			—41.7%	—33.0%	—12.6%	—7.3%

Explanations as for Table 1

2. *Qualitative*

An analysis of the qualitative aspect of the experiments revealed a considerable difference between the anterior levels (segm. V-IX) and the posterior one (segment XII).

a. Level of intersegments V-IX. At the level of intersegment V there were only a few slight anomalies in the experimental series. These were irregularities in segmentation or split metameres. In some cases they became less distinct towards the end of regeneration.

The anomalies in the experimental series at inters. VIII and IX were of a similar character. One specimen only showed a small excrescence on one side of the peristomium on the 14th day; this, however, was of a transitory nature and disappeared within a few days. It is worth while mentioning here that complete inhibition of regen-

eration was also of a temporary nature at the higher levels studied.

b. Level of intersegment XII. Both the experimental series as well as the controls showed anomalous regenerates. The proportion in the former, however, was considerably higher, being 50 per cent against 30 per cent in the controls.

The main characteristic of these anomalies was retarded and stunted growth. In several respects the regenerates resembled those of higher levels, arrested midway in their regrowth, due probably to lack of nutritive and growth substances. The most frequent deficiencies were: failure of development of the buccal cavity and the prostomium, both often coupled with a shortening of the anterior head segments. A few cases showed excessive shortness and underdevelopment of all regenerated segments.

Amongst the anomalies of the experimental series three were found which did not have counterparts in the controls, being characterized rather by excessive growth and monstrous appearance (Figures 2-5).

The first case (M-A 12) exhibited a tail-like appendage on the head (Figure 2) showing slight annulation when viewed with higher magnification. It was considerably larger in the earlier stages of regeneration (3rd and 4th week) and in life was trailing motionless behind the head, apparently due to lack of innervation.

The second case (M-B 12) had a similar protuberance on the mid-dorsal line (Figure 3). The irregular annulation of the rest of the regenerate was not peculiar to the experimentals only and a similar case was also observed in the controls.

The last case (M-C 12) Figures 4, 5, showed two ventro-lateral excrescences of considerable size. In earlier stages of regrowth the regenerate had the appearance of a triple head. Later the dorsal portion gradually overtook the ventral excrescences.

Histological examination disclosed the following details:

Case M-A 12. The appendage showed normal epidermis and circular musculature followed however by a network of connective tissue interspersed with some muscle fibres, both extending in all directions and filling the core of the appendage. At the base of the "tentacle" small setæ were observed. No nerves could be detected.

From the base of this outgrowth and within the first three segments extended a more or less centrally located mass of largely unorganized, though well differentiated nervous tissue. Within it several cavities

were noticeably lined with an epithelium, bearing cilia or rods. Posteriorly this mass was irregularly connected with a normal ventral nerve cord. Embryonic nephridia were found in the second segment. Further down and along the rest of the regenerate, only the digestive tract exhibited unorganized and chaotic features: it consisted of a series (up to eight in cross-section), of not interconnected vesicles or less frequently solid masses, exhibiting unmistakably typical intestinal epithelium. Wide blood lacunae surrounded them in an irregular manner.

Case M-B 12. The appendage showed a very narrow canal opening at its tip and running centrally along it. Below the base it bent backward and downward terminating near the regenerated, normal, digestive tract. As from the bend, it appeared lined with flat epithelium gradually turning sub-cylindrical at its posterior end.

Case M-C 12. The swellings were found due to extremely large blood lacunae and vessels in the ventral and particularly ventro-lateral quarters with an almost complete lack of blood vessels in the corresponding dorsal region, suggestive of a dorso-ventral inversion. Posteriorly these vessels were followed by masses of undifferentiated tissue connected with and somewhat resembling that of the ventral nerve cord. The nervous system throughout the regenerate showed a lack of differentiation of the nerve (ganglion) cells, with otherwise normal organization.

An interpretation of these malformations is rather uncertain as is often the case in anomalous growth.

The appendage of the first case though very much resembling a tail in life did not show microscopically sure proofs of such a character except perhaps for the setae. These, though not necessarily characteristic of the tail region, normally begin in the third segment and their presence here indicates a peculiarity of a lower level. The same applies to the high location of the nephridia and the mass of nerve tissue. The latter and the malformations of the gut represent in addition failure of organization though coupled with a well advanced tissue differentiation.

The appendage of the second case showed more certain caudal characteristics. The opening of the digestive tract on the dorsal side is characteristic of the tail; similarly, the whole appearance of the duct and its cells resembled those of the last posterior segments. On

the other hand conditions in the third case were definitely non-caudal and suggested an attempt to formation of supernumerary head ganglia.

D. Discussion

The above experiments offer additional evidence of the importance of the trephocytes in regeneration. They support earlier direct observations, of which the parallelism between the trophic and regeneration gradients, and the disappearance of the capacity to regenerate during the peak of sexual reproduction were the most important.

Additional evidence is gained by observations on individuals in the late embryonic stage: these possess only small head aggregates extending to segment IX and they do not regenerate heads posteriorly to this level. Experimentally the point can be carried further by injecting head trephocytes into the posterior region. So far it was possible to produce a high percentage of well differentiated heads on segment XXI where few and very rudimentary heads are normally formed.

The different outcome of the experiments in the higher and as compared with the lower level requires some further explanation. Observations indicate that in the adult the region from segms. V—VIII or IX is that which attracts mostly the trephocytes: here the main aggregate is formed and kept in shape and position by some intrinsic factor and here its cells become "cephalized," i.e., transformed into specific head trephocytes. This attracting and organizing force gradually decreases in a posterior direction and the primary aggregate appears broken up into several big fragments at its posterior end, in about 50 per cent of the cases studied. Within the limits of the secondary aggregates these forces decrease further and finally disappear. There are cytological indications that the cephalizing power decreases in a corresponding way.

All this appears to explain the failure to arrest regeneration permanently and to produce serious anomalies in the higher levels: the forces attracting, organizing, and cephalizing the trephocytes are considerable here and even numerous operations do not permanently inhibit regeneration nor produce considerable anomalies. In the lower levels, however, these ends are achieved even with a small number of operations, due to a decrease of the factors acting upon the trephocytes.

In this light the formation of monstrosities in our experiments is attributed to the failure of the trephocytes to become specifically organized and differentiated (cephalized) which in the last instance probably amounts to a disturbance in the physio-chemical interaction between these cells and those of the regenerate. The latter arise locally at the side of the cut; their source are apparently the replacement (basal) cells which are reported from most tissues of this species.

From a purely histological point of view the present findings have some similarity with conceptions frequently expressed since Randolph (1892) on the neoblasts, viz: that the regenerative power depends on the occurrence and number of these cells. The neoblasts, however, are normally immobile, mesodermal cells of an embryonic character which differentiate during regeneration into some or all tissues. Their functional equivalents in Eisenia are apparently the replacement or basal cells. It has become the practice in recent years to include the latter cells with the neoblasts (see the reviews of Stolte '36, Hyman '40). Our observations indicate, however, that the basal cells do not become active unless affected by the trephocytes and the substances released by them. In contrast with the neoblasts, the trephocytes are specialized cells forming a normal constituent of circulating body fluids, and do not transform into any of the cellular components of the regenerate.

The study of the trephocytes and regeneration leads to the assumption that in this species two, mutually independent, gradients exist: a head gradient and a tail gradient. Various observations, too numerous to be dealt with here, suggest that essentially similar conditions prevail in all oligochaetes and apparently other groups of animals.

E. Summary

Removal of the head trephocytes (eleocytes) resulted in a retardation or arrest of cephalic regeneration and in formation of anomalies and monstrosities.

In higher levels inhibition was only temporary and the anomalies slight. At the lower level it was more pronounced considering the small amount of removals performed; in one case regeneration was completely arrested for the period of the experiment, the number of

anomalies was larger and, in addition, several monstrosities having no counterparts in the controls were produced.

The formation of monstrosities at the lower level is attributed to the decrease of forces organizing and cephalising the trephocytes which apparently amounts to a disturbance in the physio-chemical interaction between these cells and those of the regenerate. The latter are of local origin arising from replacement (basal) cells. No neoblasts are found in this species.

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SEASONAL CHANGES IN OBELIA COLONY COMPOSITION*

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"Nothing has such power to broaden the mind as the ability to investigate systematically and truly all that comes under thy observation in life." *Meditations* of Marcus Aurelius, ii, 11.

A. INTRODUCTION

Our use of Obelia for the systematic exploration of the part played by amino acids in developmental growth occurred over the period from April to September. During the seven years from 1933 to 1939 data were accumulated on nearly a million hydranths carried by close to sixty thousand colonies. Record was made of the developmental status of each hydranth at time of collection. It is from this record that the present study is made. Record was also made of the subsequent growth of each hydranth. This will be the subject of later analyses.

Over these years the impression kept cropping up that colonies collected in the Spring had more buds than those of later months. And it seemed as if more animals went on to the senile state in Summer than at other times. These impressions suggested that colony composition is season-factored. Inquiry was indicated.

First—because change in colony composition is evidence of change in colony growth activity. And knowledge of how colony composition changes with season could aid in understanding of how and what influence season factors may have on colony growth activity.

Second—because colony growth reaction to experimental procedure is tied in with colony growth activity at time of beginning experiment. Hence knowledge of season change in colony compo-

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sition could help in evaluation of experimental results obtained at different times of year.

And finally—because any such record of season change contributes to knowledge of how the processes of living are integrated into the broad sweeps of universal forces. Such knowledge has both cultural and pragmatic values.

A bit of clarification is in order for those less familiar with this material and with the broad underlying principles of growth expression.

Obelia colonies are colonies of hydranths. These exhibit distinctive structural states which are definitive of a dominant subtending activity (Hammett, 1943).

The colonies produce gonangia. These also exhibit distinctive structural states. But they are not as definitive of a subtending activity as are those of the hydranths. And the gamut of activities they stand for is less extensive. Thus the activities of maintenance, regression, catabolism, and recurrent growth are not clearly distinguishable. At least during the period of observation used here.

These things being so, Obelia colony composition is taken as composed of hydranth buds, cones, cylinders, complete betentacled organisms, shrunken senile animals, and bare and broken pedicels. It is these which provide the basic data for study of seasonal change in colony composition. The gonangial data are supplementary.

The percentage distribution of the several hydranth states in the hydranth population as a whole is the measure of colony composition. Changes in these percentages are indices of the extent and kind of change in colony composition.

Thus the colonies of the first week of May, 1933 (line A), have one composition; those of the first week of August, 1933 (line B), have another (from Table 5).

o/o	Buds	Cones	Cyls.	Compls.	Senils	Empties	Brkns.	Total
A	22.8	3.6	8.7	56.2	1.8	5.1	1.8	100.0
B	10.7	2.5	3.6	80.5	1.0	1.0	0.6	99.9

It has been stated that change in colony composition is evidence of change in colony growth activity. Buds are the product of an antecedent growth activity. So when the composition of colonies changes from 22.8 per cent buds in May, to 10.7 in August this is

evidence of a change in *expression* of the subtending growth activity which here is Initiation. And buds are the antecedents of cones. The which are also produced by growth activity. So the change in composition with respect to buds from May to August is also evidence of change in *potentiality* for expression of the succedent growth activity. Which here is Proliferation. And so on similarly for the other structural states exhibited by Obelia colonies at time of collection.

Thus colony composition is index both to the immediately antecedent and the immediately succedent growth activity of the colony components—singly and collectively. And season change in colony composition is index of season change in colony growth activity.

The idea of correlation between growth reaction to experiment and growth activity at time of beginning experiment has factual basis. Thus the older the rat at time of thyroid removal the greater the retardation of subsequent growth. This holds for body and organ weights, and body, tail and bone lengths.[2] Therefore since growth activity decreases with age these data show that growth reaction to experiment is correlated with growth activity at time of beginning experiment.

No test of this has been made with respect to Obelia growth. But colonies in May do have more buds than in August. This means that May colonies are set to produce more buds than those of August. It is known that reaction to experiment is conditioned by the state of preparedness of the reactant. So the reaction of bud production to experiment in May could differ from that in August. And since bud production is a growth activity which is integrated into that of the colony as a whole, it is evident that colony growth reaction to experiment may be conditioned by colony growth activity at time of beginning experiment. From which it follows that since colony composition is index of colony growth activity, knowledge of season change in colony composition could help in evaluation of colony growth reaction to experiment done at different times of year.

So much for the composition background. Now for the season. Though season is a waste-basket word of loose usage it has a

[2]To save space references to the 10 or more reports on the work are omitted. They may be found in the *Am. J. Anat.*, *J. Comp. Neurol.*, *J. Exp. Zoöl.*, and *Am. J. Physiol.*, for 1926-1927.

precise frame of reference in the interval between a solar equinox and a solar solstice. This is basic because the four such intervals are essentially everywhere the same on earth while the other season phenomena occurring during a given interval vary from region to region.

According to the *Old Farmer's Almanac* the boundaries of the four intervals for 1943 were: from the vernal equinox on March 21 to the northern solstice on June 22; from the northern solstice to the autumnal equinox on September 23; from the autumnal equinox to the southern solstice on December 22; and from the southern solstice to the vernal equinox on March 21. We take this astronomical delimitation as season-defining and primary. All other terrestrial change characteristic of a given interval as season- associated and secondary.

Season associated phenomena are separable into factors and the factored. Here Obelia colony composition is the factored and light, temperature, salinity, pH, and plankton the factors.

Light: The growth and activities of most organisms are affected by changes in duration and intensity of daytime illumination. Over the period of our collections Obelia colonies were exposed to increasing light from April to late June, and to decreasing light from late June to late September. Light is a season-associated variable of possible influence on Obelia colony composition.

The light affect is compounded of duration and intensity. We have record of the former; but not of the latter. Obelia colonies are alternately exposed and submerged by tidal ebb and flow. The depth and duration of submersion varies, and the hour of exposure changes from day to day. Combining this with the fact that intensity-conditioning variations in cloudiness are local in character it is seen that any estimate of this as a factor in season influence from other than local records would be futile.

Temperature: The activity and growth of all organisms is factored by temperature. Temperature is a season-associated variable. Over the period of our collections it increases from April to mid-August and then begins to decrease. We have continuous record of local atmospheric temperature throughout the years of our studies.

Salinity: There is evidence that salinity may factor the growth of marine organisms, and that it is seasonally variable (Sverdrup, Johnson, and Fleming, 1942; Flattely and Walton, 1922; Johnstone, 1908). We have no record of local changes in sea-water salinity.

But note of possible variations in relation to Obelia colonies is apropos. Their habitat on the under-side of rocks is exposed twice a day at low tide. Evaporation undoubtedly increases the salinity of the water left clinging to the colonies. So seasonal change in light and temperature could produce seasonal change in salinity of the sea-water immediately in contact with the colonies during their period of non-submersion. Furthermore seasonal differences in local fresh-water drainage and rainfall could change the salinity of the waters of immersion. Absence of records makes analysis impossible.

pH: Sea-water is a weakly buffered solution of narrow pH range. Changes within this range are accompanied by changes in growth expression, and vice versa (Hammett and Rivard, 1940; Sverdrup, Johnson, and Fleming, 1942). There is no good evidence of a general seasonal trend in sea-water pH. This may be because plankton activity is a major determinant of pH change through its influence on sea-water CO_2 concentration. And plankton is highly variable. Light and temperature changes induce changes in plankton activity; wind and current shift the result from region to region; and there is change in kind (Sverdrup, Johnson, and Fleming, 1942; Flattely and Walton, 1922; Johnstone, 1908). This does not mean that trends may not exist. Such are conceivable in regions where plankton influence is non-erasive; and in localities so restricted that plankton influence is seasonally regular.

A short series of daily pH determinations made on the eulittoral waters of our shore from June 13 to September 10, 1941, is instructive. These are records of water from Cape Cod Bay opposite the Provincetown hook. Free of sewage and manufacturing wastes the waters come in over sand flats exposed in great area at low tide to a gradually sloping sandy beach. Tidal depth ranges from eight to thirteen feet. There is no vegetation on the flats—the eel-grass having long since gone——but the tide covers a ten-foot-wide strip of stones at the beach-flat junction which is a breeding place for Ulva, Littorina, and Balanus. There are scattered small springs of fresh water welling up on the mid-beach.

The samples came from water three or more feet in depth. They were collected between eight and nine in the morning, twelve and one at mid-day, and four and five in the afternoon. A new clean white-enamel bucket was used. This was rinsed in the Bay five times

before use, and cleaned with fresh-cut, sterile, unmedicated surgical gauze and five rinsings in the Bay after use. The bucket was not used for any other purpose. Mr. Donald Rivard made the pH determinations with a Beckmann pH-meter and glass electrode. The data, which have not been published, were segregated in part by Mr. S. S. Chapman. They comprise 230 readings taken on 80 of the 89 days from June 13 to September 10 inclusive. The nine days of no readings were mostly Sundays. On five of the 80 days readings were omitted at mid-day and evening.

Preliminary plottings gave indication that morning pH was less than mid-day and afternoon; that high-tide water had a lower pH than low-tide; and that there was a drift to lower values in September than in June. The trends are brought out by segregation.

Table 1 is a frequency distribution of the direction of difference between morning, mid-day and afternoon pH. The figures are for the combined data of four consecutive groups of 20 days each. The first horizontal bracket compares morning and mid-day; the second, morning and afternoon; and the third, mid-day and afternoon. The first section gives the frequency; the second the percentage distribution. A difference of 0.02 pH was taken as indicative. Such was present in all but 13 of the 230 comparisons.

The data show an increase in pH during daylight hours, viz., morning pH was less than mid-day; morning was less than afternoon; and mid-day was less than afternoon.

Increase in sea-water pH is usually a matter of decrease in CO_2. Decrease in CO_2 is a usual accompaniment of increase in phytoplankton activity. And sunlight being a stimulus to photosynthesis, is a stimulus to phytoplankton CO_2 consumption. The day-time rise in pH recorded here is therefore attributable to CO_2 decrease resulting from daylight stimulation of phytoplankton photosynthesis. Even though we have no measurements either of CO_2 or of plankton activity.

The seriatim charting showed some recurrent dips in morning pH. Since these occurred every two weeks—more or less—an association with tidal rhythm was suspected. So a table (Table 2) was drawn up in which actual readings on morning *low-tide* water are compared with those from the *next* morning *high-tide*. And so on similarly for mid-day and afternoon readings. The data cover the tidal range

TABLE 1

COMPARING MORNING, MID-DAY, AND EARLY EVENING pH OF EULITTORAL SEA-WATER OF CAPE COD BAY AT NORTH TRURO, MASSACHUSETTS FROM JUNE 13 TO SEPTEMBER 10, 1941

Quartile Dates	Morning vs. Mid-Day 8-9 A.M. vs. 12-1 P.M. Number +	—	=	% +	—	=	Morning vs. Early Evening 8-9 A.M. vs. 4-5 P.M. Number +	—	=	% +	—	=	Mid-Day vs. Early Evening 12-1 P.M. vs. 4-5 P.M. Number +	—	=	% +	—	=
6/13-7/4	1	14	4	*5.3*	*73.7*	*21.0*	0	17	1	*0.0*	*94.4*	*5.6*	5	13	0	*27.8*	*72.2*	*0.0*
7/5-7/26	4	13	2	*21.0*	*68.5*	*10.5*	2	17	1	*10.0*	*85.0*	*5.0*	5	12	2	*26.3*	*63.2*	*10.5*
7/28-8/19	1	19	0	*5.0*	*95.0*	*.0.0*	0	19	0	*0.0*	*100.0*	*0.0*	6	12	1	*31.5*	*63.2*	*5.3*
8/20-9/10	6	10	2	*33.3*	*55.6*	*11.1*	1	16	0	*5.9*	*94.1*	*0.0*	2	15	1	*11.1*	*83.3*	*5.6*
Totals	12	56	8	*15.8*	*73.7*	*10.5*	3	69	2	*4.1*	*94.2*	*2.7*	18	52	4	*24.3*	*70.3*	*5.4*

TABLE 2

THE LOW AND HIGH TIDE pH OF EULITTORAL SEA-WATER, CAPE COD BAY, NORTH TRURO, MASSACHUSETTS FROM JUNE 13 TO SEPTEMBER 10, 1941

Morning 8-9 A.M.				Mid-day 12-1 P.M.				Early evening 4-5 P.M.			
Low tide		High tide		Low tide		High tide		Low tide		High tide	
Date	pH	Date	pH	Date	pH	Date	pH	Date	pH	Date	pH
6/13	8.28	6/19	8.05	6/16	8.31	6/23	8.22	6/23	8.25	6/16	8.38
14	8.27	20	7.98	17	8.28	24	8.22	24	8.30		
		21	7.97	18	8.28	25	8.27				
						26	8.20	Av.	*8.28*	Av.	*8.38*
Av.	*8.28*	Av.	*8.00*								
				Av.	*8.29*	Av.	*8.23*	7/ 7	8.21	6/30	8.22
6/27	8.15	7/ 4	8.01					8	8.16	7/ 1	8.19
28	8.22	5	8.03	7/ 2	8.20	7/ 8	8.16				
29	8.19	6	8.03	3	8.19	9	8.20	Av.	*8.19*	Av.	*8.21*
30	8.19					10	8.37				
								7/21	8.20	7/14	8.16
Av.	*8.19*	Av.	*8.02*	Av.	*8.20*	Av.	*8.24*	22	8.21	15	8.08
								23	8.26		
7/11	8.22	7/17	8.02	7/16	8.03	7/22	8.08				
12	8.19	18	8.09	17	8.02	23	8.00	Av.	*8.22*	Av.	*8.12*
		19	7.98	18	8.18	24	8.14				
						25	8.40	8/ 6	8.28	7/29	8.22
Av.	*8.21*	Av.	*8.03*			26	8.18	7	8.15	30	8.20
										8/ 4	8.30
7/26	8.18	8/ 4	7.90	Av.	*8.08*	Av.	*8.10*				
28	8.19	5	7.94					Av.	*8.22*	Av.	*8.24*
				8/ 1	8.14	8/ 7	8.16				
Av.	*8.19*	Av.	*7.92*	2	8.18	8	8.14	8/19	8.10	8/12	8.10
								20	8.20	13	8.26
8/ 9	8.10	8/18	8.10	Av.	*8.16*	Av.	*8.15*	21	8.22	14	8.30
11	8.09	19	7.96					22	8.25	15	8.23
12	7.96			8/14	8.20	8/20	7.94				
13	8.12			15	8.21	21	8.16				
				16	8.16	22	7.98	Av.	*8.19*	Av.	*8.22*
Av.	*8.07*	Av.	*8.03*			23	7.80				
						26	8.22	9/ 3	8.23	8/28	8.21
8/25	8.14	9/ 1	7.97					5	8.20	29	8.27
26	8.12	2	8.12	Av.	*8.19*	Av.	*8.02*				
27	8.04							Av.	*8.22*	Av.	*8.24*
28	8.25			8/30	8.22	9/ 4	7.92				
29	8.25					5	8.09				
						6	8.18				
Av.	*8.10*	Av.	*8.05*			8	8.22				
9/ 9	8.17			Av.	*8.22*	Av.	*8.10*				
10	8.16										
Av.	*8.17*										

from one and a quarter hours before and after dead low and high water. Vagaries in number of observations in a given set derive from variability in length of tidal period, hour of collection, and records.

The data show that the pH of high-tide water was less than that of low-tide in the morning; that this difference decreased in degree and consistency by mid-day; and that by afternoon the situation was reversed, viz., pH of afternoon high-tide water tended to be greater than that of low. While these latter differences are small, their distribution and the fact that pH did rise generally during the day allow the assumption that the shift is real.

Since high-tide pH starts lower than that of low-tide; rises towards the latter by mid-day and passes (sic) it in the late afternoon it appears as if the day-time rise in pH is greater in high than in low waters. While explanation in terms of phytoplankton population and photosynthesis activity might be had, the lack of informative data make such too speculative for present pertinency.

Turning now to the third drift, Table 3 gives the frequency with which pH fell in the higher 8.20-8.42 and the lower 7.80-8.19 range during eight successive 10-day periods from June 13 to September 10. Dates are in the top line. The upper section gives the high pH incidence, the lower the low pH incidence for morning, mid-day, and afternoon separately. The first column of each period is the absolute incidence; the second the percentage. The sum of the first column corresponding numbers is the number of readings made during a stated period at a stated time of day. Thus from June 13 to 24 there were 10 mid-day readings; nine high, one low. Or 90 per cent were high; 10 per cent were low. And so on similarly. The bottom lines of each section give the total incidence in the stated pH range for each period; together with the percentage distribution between the two ranges. Thus from June 13 to 24 there were 22 high pH readings; 7 low. Or 76 per cent were high; 24 per cent low. And so on similarly.

The table gives added proof of daytime rise pH. Thus high pH was found *less* often in the morning than at mid-day or afternoon. And *less* often at mid-day than in the afternoon. Conversely low pH was found *more* often in morning water than in mid-day or afternoon. And *more* often at mid-day than in the afternoon. Finally, morning pH was low more often than it was high, and afternoon was high more often than it was low.

Evidence of season drift is as follows: High pH incidence drops from 22 (or 76 per cent) in the first June period to 10 (or 38 per cent) in the September period. And low pH incidence rises from 7

TABLE 3

SEASON CHANGE IN MORNING, MID-DAY AND EARLY EVENING *p*H OF EULITTORAL SEA-WATER OF CAPE COD BAY AT NORTH TRURO, MASSACHUSETTS FROM JUNE 13 TO SEPTEMBER 10, 1941

Date	6/13-24		6/25:7/4		7/5-15		7/16-26		7/28:8/7		8/9-19		8/20-30		9/1-10		Tot.	Per cent	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	Within range	Of Tot.
Coll.	pH 8.2-8.42																		
A.M.	4	*40*	4	*40*	1	*10*	0	*0*	0	*0*	0	*0*	*2*	*20*	0	*0*	11	10	14
Noon	9	*90*	7	*78*	5	*56*	1	*10*	4	*40*	3	*30*	4	*40*	4	*50*	37	35	49
P.M.	9	*100*	8	*89*	7	*70*	7	*70*	8	*80*	6	*67*	8	*89*	6	*75*	59	55	80
Tot.	*22*	*76*	19	*68*	13	*45*	8	*27*	12	*40*	9	*31*	14	*48*	10	*38*	107	100	47
Coll.	pH 7.80-8.19																		
A.M.	6	*60*	6	*60*	9	*90*	10	*100*	10	*100*	10	*100*	8	*80*	10	*100*	69	56	86
Noon	1	*10*	2	*22*	4	*44*	9	*90*	6	*60*	7	*70*	6	*60*	4	*50*	39	32	51
P.M.	0	*0*	1	*11*	3	*30*	3	*30*	2	*20*	3	*33*	1	*11*	2	*25*	15	12	20
Tot.	7	*24*	9	*32*	16	*55*	22	*73*	18	*60*	20	*69*	15	*52*	16	*62*	123	100	53

(or 24 per cent) to 16 (or 62 per cent) during the same interval. The trends are seen in the morning, mid-day, and afternoon data as well as in the totals. Only a falling pH could give this picture.

The progressive decline reaches bottom in the July 16-26 period. From then on there are fluctuations. The increases are correlatable with marked increase in phytoplankton in the collected samples; the decreases with marked diminution or absence thereof. The upsurges in pH may thus derive from increased CO_2 consumption incident to increase in phytoplankton population. It is to be noted that this does not bring pH back to the early level. The overall picture is one of season drift to lower pH.

It could be due to progressive increase in CO_2. Such might come from seasonal increase in number and mass of sand-flat zooids, and the schools of fish which invade this partially land-locked Bay. Regardless of cause the data indicate that pH may be a season-associated variable of possible influence on Obelia colony composition. But since sea-water pH is a low-order variable subject to other interdependent variables, and since these can vary from year to year, the fact of seasonal pH drift in 1941 is no proof such is of annual occurrence. Records for several years are needed.

To summarize: The pH of our eulittoral waters during the interval from June 13 to September 10, 1941, showed a daytime rise; a high-low-tide differential; and a seasonal drift to lower levels.

These differences are not attributable to temperature effect on pH determination. First because such is compensated for in the apparatus. Second because high t° gives low pH in buffered solutions (Clark, 1928) and there was daytime rise in pH despite daytime rise in t°. And finally because no correlation is evident between change in average temperature of the water at the dated periods and period change in high and low pH distribution.

Plankton: Although no records were made it is a fact that for the past 14 years the waters collected in spring and early summer had less phytoplankton than those of later weeks. Phytoplankton is here a season-associated variable.

Mass growth is usually correlatable with the amount of available food-stuff. And Obelia hydranths ingest plankton which is digested and used for subsistence and growth (Chapman, 1937; Hammett and

Chapman, 1938). So plytoplankton may be taken as a season variable factor of influence on Obelia colony composition.

To Summarize: The basis of Obelia colony composition resides in the seven definitive structural states of the hydranth life-cycle. Colony composition is index of colony growth activity, —antecedent and potential—and season change in composition is index of season change in growth activity. Since colony growth reaction to experiment may be conditioned by colony growth activity at time of beginning experiment, knowledge of season changes in the latter may help in evaluation of results obtained at different times of year.

Using changes in earth-sun relations as season-defining all other terrestrial phenomena or annual recurrence are taken as season-associated. Such are separable into factors and the factored. Here Obelia colony composition is the factored; light, temperature, salinity, pH, and plankton the factors. Adequate record is had of light duration and temperature. But for light intensity, salinity, pH, and plankton we have only ignorance, inference, a single series of measurements, and gross observation respectively.

The influence of these major factors is limited by conditions peculiar to the Obelia habitat, the local land-sea relations, and by their interdependability. While it may be true that each factor has a direct influence on Obelia colony composition, it is also true that the reaction of colony composition to any single factor is modified by the affectiveness of each of the other factors. Furthermore: the extrinsic affectivity of any given factor is conditioned by its subjection to the influence of the others for these factors comprise a group of interdependent variables. Thus temperature and plankton activity are directly influenced by light; salinity, pH, and plankton by temperature; pH and plankton by salinity; salinity and plankton by pH; and salinity and pH by plankton. So season differences in colony composition should be examined not only from the point of view of the relative effect produced by each factor; but also from the point of view of season change in the affectivity of each factor as effected through its interdependence with season change in the others.

B. Material and Methods

1. *Habitat*

Collections began in April or May and continued into September during the years from 1932 to 1939 inclusive. The data for 1932 are

omitted because of sparseness and discontinuity. The 1933 collections were made from rocks on the Truro shore. After that the Provincetown West End Breakwater was the source of supply because of need for larger numbers.

Up to July, 1939, the colonies came from down-hanging communities on the underside of rocks. But in the last few weeks of the last year of systematic collection it was necessary to change from rock-grown to Fucus-grown colonies because repeated cropping had depleted the former to a level below that needed for our work.

The Fucus-borne colonies grow singly like trees in a grove. The rock-borne grow in clusters, usually on barnacles. The receding tide leaves them as goat-beard-like communities from 5 to 10 mm. at base, and from 10 to 20 mm. in length. They grow more abundantly in the shade because sun and wind destroy them by desiccation at low tide. They cover the undersurfaces of rocks in the break-water caves like a deep-pile carpet because here there is protection not only from direct sunlight, but also from injury by waves and grinding sand. By mid-September, however, the cave-living colonies are usually infested with algae. So it is usually impossible to obtain sufficient for extended experimentation. That is why we have no records of Autumn colony composition.

Seasonal decline is apparently of common occurrence. McDougall (1943) reports that *Obelia commissuralis* cannot be found at Beaufort, North Carolina, after the middle of June, and Grave (1933) records disintegration and poor colonies at Woods Hole after July sets in.

Persistence in our region is largely a matter of protective habitat as described earlier (Hammett, 1943).

2. *Collection*

Test material of similar quality is a *sine qua non* for a long-term experimentation. So selection is necessary. The data presented here are not from random samples, but from colonies chosen on the basis of qualities which early experience indicated as desirable. The clusters were selected at time of collection, and the colonies were selected from the collections at time of setting up experiment.

When Obelia-bearing barnacles are collected without discrimination into a finger-bowl filled with sea-water, two extremes of quality may

be distinguished as the colonies fan out from their attachments. On some the colonies are thin, pale, and fragile appearing. On others they are stocky, yellowish, and sturdy. The hydranths of the former tend to transparency. Those of the latter stand out as whitish knobs. These are probably the better feeders (Hammett and Chapman, 1938). The thin colonies float out languidly from the barnacles. The stocky stand out stiffly. The microscope shows these to be better nourished. Their hydroplasm fills the hydrocaulus more fully. They are stronger, because more resistant to injury on manipulation. And they are more vigorous because their tentacle activity is brisker. Such as these were consistently selected for our work.

The barnacle-borne clusters in their natural habitat reflect the quality of the constituent colonies. Clusters which are long, limp, watery, palish, slick, and wan are composed largely of weaker colonies. Clusters which are firm, compact, dryish, light tan in color, and not over 20 mm. long are composed of the stronger colonies. These are the ones collected. Granulation comes from full-fashioned hydranths; slickness from scrawny. No written description can take the place of comparative observation, trial and error, experience and some intelligent apperception.

The collector who really wants suitable material must be willing to squeeze and squirm on back or belly through narrow wet crevices to get where the good little colonies grow. He will get cut by barnacles and soaked by the slimy algae. He may have to squat in a foot or more of water as he collects from the rock above. And August brings the pestiferous nit. It is no job for the fastidious; for those who fret over cuts and scratches; or for those disinclined to exertion. It is a good test of character.

Once a suitable plantation is found, collection is simply a matter of scraping the colony-bearing barnacles from the rock with a putty-knife and catching them in a pint fruit-jar filled with sea-water. The colonies are fragile and to be treated with respect. As far as we are concerned they are never touched by human hand. Damage from the putty knife is avoided by keeping it forward edge flat-tight against the rock as the barnacles are disengaged.

When the jar is two-thirds full it is emptied into sea-water in a four-quart white-enamel bucket. The jar is immersed in the water, tilted, and the barnacles allowed to slide gently out into the larger receptacle.

The amount collected depends on the number of workers using the colonies and the proportion of suitable colonies in the clusters. Check on this is made on finger-bowl samples before and during collection. Once the required amount is obtained one crawls from under the rocks and transfers the collection to a 12-quart white-enamel bucket full of sea-water using the same procedure as already described. Flotsam is gotten rid of by pouring the water—not the barnacles—from the pail and gently refilling by submersion in a convenient pool. The collection is then taken to the laboratory, the water poured off, and the pail again filled with freshly collected sea-water.

These water changes serve to get rid of a considerable amount of barnacle detritus which might affect if not harm the colonies. Moreover the maintenance activity of the tens of thousands of hydranths could conceivably so change the CO_2 concentration that the pH would reach a level nocious to the colonies on long exposure. To minimize these potential dangers we practice, and advocate the practice of, the indicated changes; transportation in abundant sea-water; removal of colonies from crowded and contaminating environment as soon as possible; and keeping the collection out of direct sunlight.

The collecting utensils are used for no other purpose whatsoever. They are kept scrupulously clean by repeated scrubbing and rinsing in sea-water before and after use. No other water is used. White-enamel pails are the containers of choice because they yield a minimum of metallic ions and growth-affecting solutes. They are discarded once the enamel breaks.

3. *Selection of Colonies*

The kind has been described. The procedure is as follows. Barnacles with the better colonies are picked one-by-one from the collection, using the finger-bowl technic noted above. Clusters of suitable quality are segregated six or seven to the bowl. Others are thrown away. All handling is with tweezers. Each barnacle is picked up by its base, not by its colonies.

Once in a bowl the fanned-out colonies are snipped off close to the barnacle with iridectomy scissors. They are then transferred one-by-one to clean freshly collected sea-water in another finger-bowl. This gets them away from barnacle effluvia. Glass medicine droppers are used for all transfers. These have been de-tipped to

give a wide mouth, the edge of which is fused smooth to prevent cutting. The rubber nipple is washed free of powder before use. The colony is most gently sucked into the pipette with plenty of water and as gently expelled into its new solution as the pipette mouth is held well under water. Forceful intake and expulsion of colony and/or water is just not done.

Reliable results depend here as elsewhere as much on proper handling as on proper selection of test material. But here as elsewhere it is impossible to put across to the reader all the minutiae which distinguish good technic. About all that can be done is to emphasize the necessity of always keeping in mind the fact that hydranths and gonangia are delicate and fragile; that their integrity is to be respected; and that no technic is good technic which leads to their destruction. So much for general procedure.

The colonies used for this estimate of season change were not collected for that purpose. They were collected for use in growth experiments with amino acids. Description of the particular procedure employed in their selection is therefore necessary in order that clear knowledge and no misconception of the nature of the material may be had.

Two sets of 15 each were selected—a test and a control—of approximately the same growth potentiality. This was done by taking the colonies one-by-one into a Syracuse watch-glass with plenty of sea-water and mapping colony composition as seen at 30 magnification under a dissecting microscope. Such a map gives both the number and the location on the hydrocaulus of each of the definitive structural states described and pictured elsewhere (Hammett, 1943). First a control colony; then a test.

As selection proceeds the colonies of one set are matched with those of the other with respect to colony size (number of hydranths per colony and branching); and with respect to the number of buds and complete hydranths per colony. The end-result is two sets each having approximately the same number of colonies, hydranths, buds, and complete organisms. An example of one such set-up made by one worker is given in Table 4. Section *A* shows that selection gave two sets with the same number of colonies in each and a like distribution of colony size based on number of hydranths per colony. Section *B* shows the total number of hydranths, buds, and complete

animals was essentially the same in each set. Thus the two were similar in composition, which is expressed in percentage figures in Section C. The two were therefore similar in growth potentiality.

Matching is accomplished by keeping count of the accumulating number of buds, complete animals, and total population in each developing set. Colonies which kept these together were accepted. Those which would cause divergence were rejected, unless there was prospect of bringing about reconvergence by later selection. In all cases only colonies were used which met the standards of suitability described above. When eleven or 12 pairs had been chosen it sometimes became necessary to trim larger colonies down to size. This was done by cutting off a basal segment of the hydrocaulus with its members. There was no lopping off of hydranths or branches separately. Only bottom bits were removed. The top-two-thirds or three-fourths were left intact. Colonies were not, however, selected on any arbitrary standard of composition. Those used were, within the limitations described in the foregoing, truly representative of the composition obtaining at time of collection. As such they provide a basis for estimate of season change.

4. *Compilation*

The number of hydranths existing in each of the seven definitive structural states may differ greatly in different colonies Indeed it is unusual to find all seven represented in a single colony. But all are represented in an assembly of colonies. So colony composition for a given day, week, or period is not obtainable from the single colony, but from sets of colonies. But as Table 4 shows, even sets of colonies as made up for our work may have small numbers of certain items.

As everybody knows, the greater the number of organisms used the greater the validity of differences which may obtain between them from one time to another. It is therefore desirable to have more organisms than are present in the single set. So the data of control and test sets made on colonies as collected and before experimentation are combined.

Collections were made three times a week. On Mondays, Wednesdays, and Fridays. On most of these days in most years five set-ups

TABLE 4

EXAMPLE OF OBELIA COLONY SELECTION WHEREFROM ARE DERIVED THE DATA USED IN ESTIMATE OF SEASON INFLUENCE ON POPULATION

From set-up recorded for experiment No. 6 by James Toman on June 3rd, 1938.

A. Number of colonies and their distribution according to the number of hydranths per colony

	No. Cols.	Number of hydranths														
Control Set	*15*	14	15	15	15	16	16	16	17	18	19	19	19	19	20	20
Test Set	*15*	15	15	16	16	16	16	16	16	17	17	17	18	19	19	21

B. Total number of hydranths and the number of each of the definitive structural states. Colony composition

	Tot. No.	*Bud*	Cone	Cylinder	*Complete*	Senile	Empty	Broken
Control Set	*258*	*29*	5	20	*193*	5	1	5
Test Set	*254*	*29*	7	21	*190*	3	1	3

C. Percentage contribution of each structural state to colony population. Index of colony growth potentiality

	Total %	*Bud*	Cone	Cylinder	*Complete*	Senile	Empty	Broken
Control Set	*99.9*	*11.2*	1.9	7.8	*74.8*	1.9	0.4	1.9
Test Set	*100.1*	*11.4*	2.8	8.3	*74.8*	1.2	0.4	1.2

were made from each collection, one by each of five assistants,—or a total of 15 a week.

A week-by-week record is convenient for season study. And in the combination of 15 control and 15 test sets, or 30 in all, there is a goodly number of all items. This is shown in Tables 5 to 11.

Furthermore, by combining a week's data into one set of figures there is approach to stabilization for factors possibly productive of day-to-day variability. Such factors are day-to-day differences in duration of colony exposure to low tide before collection; differences in site of collection from the same or different rocks; daily differences in temperature; differences in salinity resulting from rain and no-rain; differences in phytoplankton, etc., etc. Study of season trend is complicated enough without introducing the colony changes imposed by these day-to-day variabilities. So the data are compiled on a weekly basis. Such are given for each of the seven years from 1933 to 1939 in Tables 5 to 11.

Lack of foresight by the Senior Author led to compilation on a calendar instead of a straight weekly record. What we have is a monthly segregation of the data into four quasi-weekly combinings.

TABLE 5

THE ABSOLUTE AND PERCENTAGE COMPOSITION OF OBELIA COLONY POPULATION FOR *1933*

The week-by-week total numbers of hydranths and colonies; and of hydranths as buds, cones, cylinders, completes, seniles, empty hydrothecae and broken pedicels; together with the average percentages of these definitive structural states in the hydranth life-cycle in the weekly population.

		Weekly totals of								Average percentages of						
Period	No. Cols.	Bd	Co	Cy	CH	Sn	Em	Br	Total	Bd	Co	Cy	CH	Sn	Em	Br
May																
1	30	63	10	24	155	5	14	5	276	22.8	3.6	8.7	56.2	1.8	5.1	1.8
2	90	162	48	76	619	11	11	6	933	17.4	5.2	8.1	66.3	1.2	1.2	0.7
3	136	255	58	122	1,038	33	31	18	1,555	15.9	3.9	7.5	67.6	2.0	1.9	1.3
4	234	435	109	204	2,095	25	28	48	2,944	14.6	3.8	6.9	71.2	0.9	0.9	1.7
June																
1	216	406	121	234	2,110	48	73	58	3,050	13.2	4.0	7.8	69.0	1.6	2.4	1.9
2	120	256	56	125	1,190	14	55	20	1,716	14.9	3.3	7.3	69.4	0.8	3.2	1.2
3	345	674	252	405	2,803	93	143	65	4,435	14.9	5.7	9.3	63.1	2.1	3.4	1.5
4	258	338	143	272	2,283	58	93	55	3,242	10.6	4.5	8.6	69.9	1.8	2.9	1.7
July																
1	291	475	151	240	2,877	23	75	33	3,874	12.4	3.9	6.1	74.4	0.6	1.8	0.8
2	270	504	224	323	2,688	73	69	32	3,913	12.9	5.7	8.2	68.7	1.9	1.8	0.8
3	270	463	196	273	2,772	61	54	57	3,876	11.9	5.1	7.1	71.5	1.6	1.4	1.5
4	492	893	289	386	4,998	69	111	54	6,800	13.1	4.3	5.7	73.5	1.0	1.6	0.8
August																
1	312	498	120	167	3,784	49	50	30	4,698	10.7	2.5	3.6	80.5	1.0	1.0	0.6
2	381	805	291	410	4,118	107	98	43	5,872	13.8	5.0	7.1	70.0	1.8	1.7	0.7
3	300	499	163	250	3,595	57	82	53	4,699	10.5	3.6	5.4	76.3	1.2	1.8	1.2
4	333	506	164	195	3,408	34	51	52	4,410	11.5	3.8	4.5	77.2	0.8	1.2	1.1
September																
1	300	639	199	280	3,379	112	127	39	4,775	13.3	4.1	5.9	70.8	2.3	2.7	0.8
2	458	507	270	379	4,482	61	96	128	5,923	8.6	4.6	6.4	75.5	1.1	1.6	2.2
3	351	345	161	196	3,163	39	95	150	4,149	8.3	3.9	4.8	76.2	0.9	2.3	3.6
4	72	184	65	98	728	16	14	13	1,118	16.5	5.8	8.7	65.2	1.4	1.3	1.2
Total	5,259	8,891	3,106	4,659	52,285	988	1,370	959	72,258							

TABLE 6

THE ABSOLUTE AND PERCENTAGE COMPOSITION OF OBELIA COLONY POPULATION FOR *1934*

The week-by-week total numbers of hydranths and colonies; and of hydranths as buds, cones, cylinders, completes, seniles, empty hydrothecae and broken pedicels; together with the average percentages of these definitive structural states in the hydranth life-cycle in the weekly population.

Period	No. Cols.	Weekly totals of Bd	Co	Cy	CH	Sn	Em	Br	Total	Average percentages of Bd	Co	Cy	CH	Sn	Em	Br
May																
3	220	498	122	193	1,887	25	65	34	2,824	17.1	4.4	7.2	67.1	0.9	2.2	1.3
4	336	709	247	313	3,316	64	83	70	4,802	14.9	5.0	6.4	69.0	1.5	1.7	1.4
June																
1	570	1,281	374	509	5,837	66	117	67	8,251	15.5	4.6	6.2	70.8	0.8	1.3	0.8
2	420	822	259	352	4,686	77	85	152	6,433	12.7	3.9	5.5	72.9	1.2	1.3	2.4
3	450	735	250	391	4,915	125	99	58	6,573	11.2	3.9	5.9	74.8	1.9	1.5	0.9
4	451	684	250	344	4,921	123	170	127	6,619	10.4	3.9	5.2	74.2	1.8	2.6	2.0
July																
1	390	652	230	396	4,466	66	124	92	6,026	10.9	3.9	6.5	73.9	1.1	2.1	1.6
2	360	684	273	361	4,423	88	183	93	6,105	11.3	4.6	5.9	72.1	1.5	3.1	1.6
3	600	1,210	368	502	7,633	82	135	156	10,086	12.0	3.6	4.9	75.7	0.8	1.4	1.6
4	450	791	361	477	5,578	59	192	133	7,591	10.5	4.8	6.2	73.4	0.8	2.6	1.8
August																
1	450	886	253	386	5,314	105	172	165	7,281	12.2	3.6	5.3	72.8	1.4	2.5	2.3
2	568	1,134	365	605	6,376	121	219	152	8,972	12.7	4.1	6.7	71.1	1.3	2.5	1.7
3	450	901	244	423	5,312	135	193	140	7,348	12.2	3.4	5.7	72.3	1.8	2.6	2.0
4	600	1,164	510	561	7,064	133	191	142	9,765	11.9	5.3	5.7	72.3	1.3	2.0	1.5
September																
1	450	938	315	406	5,473	123	133	168	7,556	12.3	4.2	5.4	72.5	1.6	1.8	2.3
2	450	1,029	421	443	5,383	99	141	79	7,595	13.5	5.6	5.7	70.9	1.3	1.9	1.1
Total	7,215	14,118	4,842	6,662	82,584	1,491	2,302	1,828	113,827							

TABLE 7

THE ABSOLUTE AND PERCENTAGE COMPOSITION OF OBELIA COLONY POPULATION FOR *1935*

The week-by-week total numbers of hydranths and colonies; and of hydranths as buds, cones, cylinders, completes, seniles, empty hydrothecae and broken pedicels; together with the average percentages of these definitive structural states in the hydranth life-cycle in the weekly population.

Period	No. Cols.	Weekly totals of Bd	Co	Cy	CH	Sn	Em	Br	Total	Average percentages of Bd	Co	Cy	CH	Sn	Em	Br
May																
1	178	446	92	163	1,641	43	20	59	2,464	17.9	3.9	6.7	66.4	1.8	0.8	2.6
2	328	823	249	391	3,767	82	69	182	5,563	15.1	4.4	6.9	67.3	1.4	1.2	3.6
3	376	1,164	292	473	3,865	95	113	145	6,147	19.2	4.6	7.5	62.8	1.5	1.9	2.5
4	554	1,275	407	693	6,013	183	160	640	9,371	13.7	4.3	7.4	64.0	1.9	1.7	7.1
June																
1	420	913	298	479	4,652	168	141	472	7,123	12.8	4.2	6.7	65.2	2.4	2.0	6.8
2	450	821	248	438	5,298	178	147	272	7,402	11.0	3.4	5.9	71.5	2.4	2.0	3.9
3	450	911	328	428	5,498	165	143	287	7,760	11.7	4.2	5.5	70.7	2.1	1.9	3.8
4	420	870	276	501	5,260	126	92	128	7,253	11.9	3.8	6.9	72.5	1.7	1.3	1.8
July																
1	600	1,286	407	697	7,181	223	312	247	10,353	12.4	3.9	6.7	69.4	2.2	3.0	2.4
2	450	858	240	514	5,423	191	144	177	7,547	11.4	3.2	6.9	71.7	2.6	1.9	2.4
3	450	640	238	472	5,813	130	153	163	7,609	8.4	3.1	6.3	76.2	1.8	2.0	2.2
4	570	805	356	558	7,279	172	217	264	9,651	8.3	3.7	5.8	75.3	1.8	2.3	2.9
August																
1	450	747	277	485	5,829	96	118	174	7,726	9.6	3.6	6.3	75.3	1.2	1.5	2.3
2	600	807	281	512	7,446	158	195	368	9,767	8.2	2.8	5.2	76.2	1.6	2.0	3.9
3	450	745	247	415	5,582	154	194	174	7,511	9.9	3.3	5.6	74.2	2.1	2.6	2.3
4	450	473	316	538	5,934	108	156	154	7,679	6.1	4.1	7.0	77.3	1.4	2.1	2.1
September																
1	450	767	280	339	5,861	76	138	202	7,663	10.0	3.6	4.5	76.5	1.0	1.8	2.7
2	675	1,544	544	746	8,744	121	134	105	11,938	12.9	4.5	6.3	73.4	1.0	1.1	0.9
Total	8,321	15,895	5,376	8,842	101,086	2,469	2,646	4,213	140,527							

TABLE 8

THE ABSOLUTE AND PERCENTAGE COMPOSITION OF OBELIA COLONY POPULATION FOR *1936*

The week-by-week total numbers of hydranths and colonies; and of hydranths as buds, cones, cylinders, completes, seniles, empty hydrothecae and broken pedicels; together with the average percentages of these definitive structural states in the hydranth life-cycle in the weekly population.

Period	No. Cols.	Weekly totals of Bd	Co	Cy	CH	Sn	Em	Br	Total	Average percentages of Bd	Co	Cy	CH	Sn	Em	Br
May																
1	240	514	133	205	3,027	31	46	43	3,999	12.9	3.3	5.1	75.7	0.8	1.2	1.1
2	360	1,029	378	430	4,222	55	51	87	6,252	16.4	6.0	6.9	67.6	0.9	0.8	1.4
3	360	964	300	406	4,430	74	57	38	6,269	15.4	4.7	6.5	70.7	1.2	0.9	0.6
4	360	1,157	302	454	4,222	40	29	29	6,233	18.6	4.8	7.3	67.8	0.6	0.5	0.5
June																
1	392	945	259	414	4,819	61	83	110	6,691	14.0	3.8	6.1	71.9	0.9	1.4	1.7
2	584	1,150	364	633	7,572	147	129	170	10,165	11.3	3.6	6.2	74.5	1.4	1.3	1.7
3	450	1,127	292	494	5,478	139	130	148	7,808	14.4	3.7	6.3	70.2	1.8	1.7	1.9
4	390	906	281	470	4,264	228	240	300	6,689	13.5	4.2	7.0	63.7	3.5	3.6	4.5
July																
1	450	986	367	665	4,690	299	340	288	7,635	12.9	4.8	8.7	61.4	3.9	4.5	3.8
2	450	830	288	570	5,494	214	261	251	7,908	10.5	3.6	7.2	69.5	2.7	3.3	3.2
3	600	1,084	432	802	7,243	234	260	285	10,340	10.5	4.2	7.8	70.0	2.3	2.5	2.8
4	600	1,120	445	795	7,344	256	327	279	10,566	10.6	4.2	7.5	69.6	2.4	3.1	2.7
August																
1	450	801	325	577	5,604	134	205	206	7,852	10.2	4.1	7.3	71.3	1.7	2.6	2.7
2	450	801	269	481	5,822	137	194	218	7,922	10.1	3.4	6.1	73.5	1.7	2.5	2.8
3	450	702	263	414	5,757	139	206	254	7,735	9.1	3.4	5.4	74.3	1.8	2.7	3.3
4	450	825	298	560	5,536	174	192	168	7,753	10.6	3.8	7.2	71.4	2.2	2.5	2.2
September																
1	450	927	292	481	5,524	168	176	150	7,718	12.0	3.7	6.2	71.6	2.2	2.3	2.0
2	600	1,055	548	878	6,891	332	301	259	10,264	10.3	5.3	8.6	67.2	3.2	3.0	2.5
Total	8,086	16,923	5,836	9,729	97,939	2,862	3,227	3,283	139,799							

TABLE 9

THE ABSOLUTE AND PERCENTAGE COMPOSITION OF OBELIA COLONY POPULATION FOR *1937*

The week-by-week total numbers of hydranths and colonies; and of hydranths as buds, cones, cylinders, completes, seniles, empty hydrothecae and broken pedicels; together with the average percentages of these definitive structural states in the hydranth life-cycle in the weekly population.

Period	No. Cols.	Weekly totals of Bd	Co	Cy	CH	Sn	Em	Br	Total	Average percentages of Bd	Co	Cy	CH	Sn	Em	Br
May																
1	384	916	208	405	3,602	89	95	121	5,436	17.0	3.7	7.6	66.1	1.7	1.6	2.1
2	436	1,066	352	411	4,311	153	138	212	6,643	15.8	5.3	6.2	64.9	2.4	2.1	3.4
3	450	1,075	308	397	5,036	140	124	200	7,280	14.8	4.2	5.4	69.2	1.9	1.7	2.7
4	538	1,638	545	644	6,335	202	144	157	9,665	17.0	5.4	6.9	65.4	2.2	1.6	1.6
June																
1	450	1,034	345	465	5,387	106	52	140	7,529	13.7	4.5	6.2	71.6	1.4	0.7	1.9
2	600	1,129	451	468	6,480	224	208	434	9,394	12.0	4.7	5.1	68.9	2.3	2.3	4.7
3	450	904	343	398	4,884	176	133	294	7,132	12.8	4.7	5.6	68.3	2.5	1.9	4.2
4	435	757	352	338	5,384	114	194	238	7,377	10.2	4.8	4.5	73.1	1.5	2.6	3.2
July																
1	600	1,071	520	611	6,769	253	298	325	9,847	10.8	5.3	6.2	68.8	2.6	3.0	3.3
2	450	978	359	467	4,975	141	185	149	7,254	13.4	5.0	6.5	68.5	1.9	2.6	2.1
3	600	1,125	413	541	7,200	161	229	274	9,943	11.2	4.1	5.5	72.5	1.6	2.3	2.8
4	450	717	322	379	5,348	178	274	192	7,410	9.7	4.4	5.1	72.1	2.4	3.7	2.6
August																
1	412	833	379	384	4,775	159	210	219	6,959	12.0	5.5	5.5	68.6	2.3	3.0	3.2
2	434	864	286	399	5,188	184	153	184	7,258	11.9	4.0	5.5	71.5	2.5	2.1	2.5
3	296	523	131	202	3,443	122	165	229	4,815	10.9	2.7	4.2	71.5	2.6	3.4	4.7
4	596	1,335	385	614	7,361	99	104	236	10,134	13.2	3.8	6.1	72.6	1.0	1.0	2.3
September																
1	442	968	239	510	5,238	121	119	87	7,282	13.3	3.3	7.0	72.0	1.7	1.6	1.2
2	450	847	280	474	5,186	135	173	204	7,299	11.6	3.8	6.5	71.1	1.8	2.4	2.8
3	450	791	304	452	5,396	121	192	198	7,454	10.6	4.1	6.1	72.4	1.6	2.6	2.7
Total	8,923	18,571	6,522	8,559	102,298	2,878	3,190	4,093	146,111							

TABLE 10

The Absolute and Percentage Composition of Obelia Colony Population for *1938*

The week-by-week total numbers of hydranths and colonies; and of hydranths as buds, cones, cylinders, completes, seniles, empty hydrothecae and broken pedicels; together with the average percentages of these definitive structural states in the hydranth life-cycle in the weekly population.

Period	No. Cols.	Weekly totals of Bd	Co	Cy	CH	Sn	Em	Br	Total	Average percentages of Bd	Co	Cy	CH	Sn	Em	Br
April																
3	360	1,044	218	496	4,620	103	51	190	6,722	15.6	3.3	7.4	68.7	1.5	0.8	2.8
4	360	1,042	210	439	4,920	93	94	150	6,948	15.0	3.1	6.3	70.8	1.3	1.4	2.2
May																
1	360	1,404	248	553	4,696	124	93	79	7,197	19.4	3.5	7.7	65.3	1.7	1.3	1.1
2	360	1,185	288	559	5,125	216	145	105	7,623	15.5	3.8	7.3	67.3	2.9	1.9	1.4
3	360	1,238	257	568	4,511	191	112	95	6,972	17.6	3.7	8.1	65.0	2.7	1.6	1.4
4	500	1,536	369	581	6,855	208	94	117	9,760	15.7	3.8	6.0	70.1	2.1	1.0	1.2
June																
1	444	1,161	243	478	5,821	155	71	129	8,058	14.5	3.0	6.0	72.1	2.0	0.9	1.6
2	450	1,119	283	468	5,564	181	96	148	7,859	14.2	3.6	5.9	70.8	2.3	1.2	1.9
3	448	1,081	296	509	5,623	227	129	164	8,029	13.3	3.7	6.4	70.2	2.8	1.6	2.1
4	298	659	150	349	3,865	153	106	164	5,446	12.1	2.8	6.4	70.9	2.8	1.9	3.0
July																
1	450	1,033	251	491	5,480	231	185	419	8,090	12.7	3.1	6.1	67.7	2.9	2.3	5.2
2	450	874	252	533	5,255	279	156	407	7,756	11.3	3.3	6.9	67.8	3.6	2.0	5.2
3	448	634	211	480	5,775	121	98	321	7,640	8.3	2.8	6.3	75.6	1.6	1.3	4.2
4	600	1,051	289	635	7,934	204	178	260	10,551	10.0	2.8	6.0	75.2	1.9	1.7	2.5
August																
1	450	718	205	389	5,846	154	99	273	7,684	9.4	2.7	5.1	76.1	2.0	1.3	3.5
2	448	784	336	421	5,631	206	181	278	7,737	10.2	3.1	5.4	72.8	2.7	2.3	3.6
3	448	896	192	433	5,744	175	121	170	7,731	11.6	2.5	5.6	74.2	2.3	1.6	2.2
4	566	1,151	239	623	7,527	173	92	146	9,951	11.6	2.4	6.2	75.6	1.7	0.9	1.5
September																
1	444	719	205	421	6,203	139	100	198	7,985	9.0	2.6	5.3	77.7	1.7	1.3	2.5
2	600	875	249	455	7,894	181	135	292	10,081	8.7	2.5	4.5	78.3	1.8	1.3	2.9
Total	8,844	20,204	4,991	9,881	114,889	3,514	2,336	4,105	159,820							

TABLE 11

THE ABSOLUTE AND PERCENTAGE COMPOSITION OF OBELIA COLONY POPULATION FOR *1939*

The week-by-week total numbers of hydranths and colonies; and of hydranths as buds, cones, cylinders, completes, seniles, empty hydrothecae and broken pedicels; together with the average percentages of these definitive structural states in the hydranth life-cycle in the weekly population.

Period	No. Cols.	Weekly totals of Bd	Co	Cy	CH	Sn	Em	Br	Total	Average percentages of Bd	Co	Cy	CH	Sn	Em	Br
April																
1	120	383	64	181	1,428	43	33	82	2,214	17.3	2.9	8.2	64.6	1.9	1.5	3.7
2	180	563	107	251	2,160	52	46	151	3,330	16.9	3.2	7.5	64.9	1.6	1.4	4.5
3	180	571	119	248	2,239	39	16	103	3,335	17.1	3.6	7.4	67.2	1.2	0.5	3.1
4	180	517	88	296	2,261	52	21	59	3,294	15.7	2.7	9.0	68.6	1.6	0.6	1.8
May																
1	180	596	106	274	2,277	47	27	44	3,371	17.6	3.2	8.0	67.7	1.4	0.8	1.3
2	180	480	114	257	2,091	56	71	67	3,136	15.3	3.7	8.2	66.7	1.8	2.3	2.1
3	178	444	125	209	2,255	87	18	55	3,193	13.9	3.9	6.6	70.6	2.8	0.5	1.7
4	240	635	169	390	3,050	116	40	80	4,480	14.1	3.8	8.7	68.1	2.6	0.9	1.8
June																
1	208	561	153	271	2,623	116	49	90	3,863	14.6	3.9	7.0	67.9	3.0	1.3	2.3
2	370	872	332	377	5,395	140	83	241	7,440	12.3	4.6	5.1	72.0	1.8	1.0	3.2
3	392	849	358	445	5,837	302	155	256	8,202	10.3	4.4	5.4	71.2	3.7	1.9	3.1
4	576	1,707	666	647	7,905	473	282	288	11,968	14.3	5.6	5.4	66.1	4.0	2.3	2.4
July																
1	294	817	311	427	3,858	255	115	155	5,938	13.8	5.3	7.2	64.9	4.3	1.9	2.6
2	448	1,476	308	374	6,689	113	36	51	9,047	16.2	3.4	4.1	74.0	1.3	0.4	0.6
3	300	924	209	266	4,096	121	37	95	5,748	16.0	3.7	4.6	71.3	2.2	0.6	1.7
4	450	1,497	364	438	5,971	127	47	89	8,533	17.6	4.3	5.1	70.0	1.5	0.5	1.0
August																
1	300	667	165	279	4,032	90	33	61	5,327	12.5	3.1	5.2	75.7	1.7	0.6	1.1
2	146	314	88	137	1,937	23	15	22	2,536	12.5	3.5	5.3	76.4	0.9	0.6	0.9
3	442	978	245	460	6,041	70	45	65	7,904	12.3	3.2	5.8	76.5	0.9	0.6	0.8
4	444	1,337	277	543	8,426	140	67	84	10,874	12.3	2.6	5.0	77.5	1.3	0.6	0.8
September																
1	448	756	191	311	6,133	106	67	174	7,738	9.7	2.5	4.0	79.3	1.2	0.9	2.3
2	152	204	66	79	1,988	35	30	87	2,489	8.2	2.7	3.2	79.9	1.4	1.2	3.5
Total	6,408	17,148	4,625	7,160	88,692	2,603	1,333	2,399	123,960							

So about 25 per cent include data for four instead of three days of collection. This however, is of no moment since season change is based on relative, not on absolute composition.

The tables give the week-by-week total numbers of colonies and hydranths; and of hydranths as buds, cones, cylinders, completes, seniles, empty hydrothecae, and bare or broken pedicels. They also give the average percentages of each in the week's population as a whole. They thus are a week-by-week record of colony composition over the designated intervals for the indicated years. As observed at time of collection. In all there were 53,056 colonies and 896,302 hydranths. The average number of animals per colony was 17. Inspection indicates that by-and-large the number of animals at any stage in any week is adequate for study of season change in colony composition.

5. *Computation*

The week's data are put together as follows: Table 12 is copy of record for the first week of July, 1936. Under "Index" we give the number of colonies, the date, the experiment number, and the initial of the assistant responsible for the set-up. Under "Number" we give the absolute numbers of each of the definitive structural states in the order of the hydranth life-cycle. Also the total number of hydranths in each set. The Control and Test sets are summed separately and recorded at the bottoms of their respective columns. These are then combined into one set of totals which is made the first line of Section *C*. The averages are given for completeness. This total of 7,635 hydranths on 450 colonies together with the total numbers of buds, cones, cylinders, complete animals, seniles, empties, and brokens is what is recorded in Table 8. And so on similarly for the other weeks in the other years of record.

These are the raw data for computation of colony composition. This is obtained by dividing the numbers of buds, cones, cylinders etc., separately of each set by the total number of hydranths of that set. These values are put down under "Percentage." Each line is thus the record of colony composition of the subtending population. These items are summed and the sums set down quite like the absolute numbers. The totals are divided by the number of sets. In this case 15 each for Control and Test percentages separately, and 30 for

TABLE 12

EXAMPLE OF OBELIA COLONY SET-UP FOR THE SINGLE WEEK FIRST WEEK IN JULY 1936

TABLE 12

EXAMPLE OF OBELIA COLONY SET-UP FOR THE SINGLE WEEK FIRST WEEK IN JULY 1936

Bd—Buds: Co—Cones: Cy—Cylinders: CH—Complete Hydranths: Sn—Seniles: Em—Empties: Br—Brokens.

	I. Index			II. Number								III. Percentage						
Cl	D	Ex	T	Bd	Co	Cy	CH	Sn	Em	Br	Tot.	Bd	Co	Cy	CH	Sn	Em	Br
									A. Control sets									
15	1	11	T	32	13	24	154	8	7	9	247	13.0	5.3	9.7	62.3	3.2	2.8	3.6
15	1	11	E	37	17	20	159	16	8	7	264	14.0	6.4	7.6	60.2	6.1	3.0	2.7
15	1	11	M	38	9	33	159	6	16	9	270	14.1	3.3	12.2	58.9	2.2	5.9	3.3
15	1	11	C	44	18	26	151	13	10	11	273	16.1	6.6	9.4	55.3	4.8	3.7	4.0
15	1	11	S	20	16	18	139	11	16	18	238	8.4	6.7	7.6	58.4	4.6	6.7	7.6
15	3	12	T	39	9	15	136	12	22	7	240	16.2	3.7	6.2	56.7	5.0	9.2	2.9
15	3	12	E	44	17	24	147	14	6	3	255	17.3	6.7	9.4	57.7	5.5	2.4	1.2
15	3	12	M	39	6	34	133	8	11	9	240	16.2	2.5	14.2	55.4	3.3	4.6	3.7
15	3	12	C	33	23	32	172	5	11	4	280	11.8	8.2	11.4	61.4	1.8	3.9	1.4
15	3	12	S	20	14	18	149	15	12	22	250	8.0	5.6	7.2	59.6	6.0	4.8	8.8
15	6	13	T	23	7	21	164	8	11	10	244	9.4	2.9	8.6	67.2	3.3	4.5	4.1
15	6	13	E	32	9	21	175	5	7	4	253	12.6	3.6	8.3	69.2	2.0	2.8	1.6
15	6	13	M	35	7	17	158	5	12	5	239	14.6	2.9	7.1	66.1	2.1	5.0	2.1
15	6	13	C	37	14	18	190	6	14	2	281	13.2	5.0	6.4	67.6	2.1	5.0	0.7
15	6	13	S	22	10	16	155	13	13	16	245	9.0	4.1	6.5	63.3	5.3	5.3	6.5
Totals:			Con	495	189	337	2,341	145	176	136	3,819	193.9	73.5	131.8	919.3	57.3	69.6	54.2
Average:			Con	33	13	22	156	10	12	9	255	12.9	4.9	8.8	61.3	3.8	4.6	3.6
									B. Test sets									
15	1	11	T	30	8	17	154	6	14	16	245	12.2	3.3	6.9	62.9	2.4	5.7	6.5
15	1	11	E	33	9	40	151	9	13	4	259	12.7	3.5	15.4	58.3	3.5	5.0	1.5
15	1	11	M	41	11	33	159	11	10	7	272	15.1	4.0	12.1	58.4	4.0	3.7	2.6
15	1	11	C	44	22	19	162	13	6	7	273	16.1	8.1	7.0	59.3	4.8	2.2	2.6
15	1	11	S	19	12	25	137	12	14	19	238	8.0	5.0	10.5	57.6	5.0	5.9	8.0
15	3	12	T	41	12	17	134	12	14	10	240	17.1	5.0	7.1	55.8	5.0	5.8	4.2
15	3	12	E	41	19	26	147	8	11	4	256	16.0	7.4	10.2	57.4	3.1	4.3	1.6
15	3	12	M	37	11	25	134	11	13	9	240	15.4	4.6	10.4	55.8	4.6	5.4	3.7
15	3	12	C	36	19	23	183	8	5	4	278	12.9	6.8	8.3	65.8	2.9	1.8	1.4
15	3	12	S	21	13	15	150	14	12	25	250	8.4	5.2	6.0	60.0	5.6	4.8	10.0
15	6	13	T	23	7	14	165	10	14	13	246	9.3	2.8	5.7	67.1	4.1	5.7	5.3
15	6	13	E	31	9	20	172	12	5	5	254	12.2	3.5	7.9	67.7	4.7	2.0	2.0
15	6	13	M	36	6	14	155	14	6	9	240	15.0	2.5	5.8	64.6	5.8	2.5	3.7
15	6	13	C	37	11	22	189	7	12	3	281	13.2	3.9	7.8	67.2	2.5	4.3	1.1
15	6	13	S	21	9	18	157	7	15	17	244	8.6	3.7	7.4	64.3	2.9	6.1	7.0
Totals:			Test	491	178	328	2,349	154	164	152	3,816	192.2	69.3	128.5	922.2	60.9	65.2	61.2
Average:			Test	33	12	22	157	10	11	10	255	12.8	4.6	8.6	61.5	4.1	4.3	4.1
									C. Control and test sets combined (A + B)									
Total:			Con+Test	986	367	665	4,690	299	340	288	7,635	386.1	142.8	260.3	1,841.5	118.2	134.8	115.4
Average			Con+Test	33	12	22	156	10	11	10	255	12.9	4.8	8.7	61.4	3.9	4.5	3.8

Cl—Number of Colonies: D—Date: Ex—Experiment Number: T—Technicians: Con—Control: Test—Test.

the combined. The results occur in the bottom line. This gives the Average Percentage of buds, cones, cylinders, etc., for the 450 colonies of record during the first week of July, 1936 (cf. Table 8). It is such as these which are given for each week of record of each year in Tables 5 to 11. And it is these which give the week-by-week record of colony composition.

Relative instead of absolute values must be used. For there are differences in numbers of the several structural states within a given set; differences between sets with respect to the number of hydranths at a given state; and differences between sets with respect to total number of hydranths. These make it necessary to bring the data to a common numerical basis if one wants to compare the contribution of one structure to colony composition with that of another, and the composition of one set of colonies with that of others. The simplest way to do this is via the percentage route. By so doing misinterpretations are avoided.

Thus Table 12 shows that the 4th Control set had more complete hydranths than the 5th (151 vs. 139). But the total number of hydranths in the 4th was greater than that of the 5th so the percentage of complete animals was actually less, viz., 55.3 vs. 58.4. The contribution of completes to colony composition was therefore greater in the 5th than in the 4th set despite the fact the absolute number was less. Direction of difference in number may not be index of direction of difference in composition.

Furthermore, equality in absolute number is no proof of equality in composition. Table 12 shows the same number (36) of buds in the 9th and 13th Test sets. But the total number of hydranths in the 9th was 278 while that of the 13th was 240. The actual contribution of buds to colony composition in set nine was less than in set thirteen, viz., 12.9 vs. 15.0. Many similar examples may be found in Tables 5 to 11. In Table 12 we also see exemplified three of the variabilities for which stabilization is approached by combinings.

There is worker-to-worker variability. The composition of a set assembled on a given day by one worker may differ from that assembled by another. Compare Control T-11 with Control E-11, etc. And this despite the fact all colonies came from the same collection, from the same rock, at the same time, on the same day. It is evident that the single set is not the most satisfactory index of

colony composition extant on a given day. A part of the difference may come from individual idiosyncrasy; most comes from chance. The larger the sample taken from the collection as a whole the more representative it is of colony composition. Hence the desirability of using all five sets when diurnal composition is of moment.

There is variation from day to day. The composition of a set assembled by a given worker on one day may differ from that assembled by the same worker on another. Compare Control T-11 with Control T-12 and T-13, etc. There is also day-to-day difference even when all five sets of a given day are combined. The average percentage of cylinders was 9.3 on July 1st, 9.7 on July 3rd, and 7.4 on July 6th. And so on similarly for other components. Extrinsic factors contributing to this day-to-day variability have already been noted. The best approximation to colony composition characteristic of a given week is obtained by combining all sets of all days. By so doing stabilization of factors productive of worker-to-worker and day-to-day variability is approached.

Finally there may be variability as between controls and tests. The composition of the test set is rarely the same as that of the control when made by the same worker from the same collection on the same day. Compare Control T-11 with Test T-11, etc. A test-control difference may even exist between the five-set diurnal combinations. The average percentage of cylinders of all controls of July 3rd is 9.7, while that of the tests is 8.4. And so on similarly for other components. Although such may be neutralized by a week's combinings, as happens here, since test composition is essentially the same as control for the week; it could happen that this would not occur. Hence a better approximation to colony composition characteristic of the period may be had by combining test and control data than by taking either alone.

This analysis added to what has gone before shows why all sets assembled by all workers on all days of the week are combined into one set of figures. The result is a reasonably good approximation of colony composition for the stated period. And since there is worker-to-worker, day-to-day, and control-to-test variability within the compass of a week's collections, it would seem as if the material is adequate for estimate of season change despite its selectivity.

The ground has now been prepared for analysis of season changes

in Obelia colony composition. For this it has been necessary to invoke the several disciplines of astronomy, physics, chemistry, mathematics, physiology, morphology, zoölogy, botany, ecology, geography, climatology, and oceanography. There are no boundaries in science; there are only interdependencies. And integration is its goal.

C. Analysis and Comment

Charts 1 to 7 visualize the week-to-week changes in percentage contribution of each of the seven definitive hydranth structures to colony composition over the stated periods for the seven years from 1933 to 1939.

The weekly averages of atmospheric Fahrenheit temperature as taken from Table 13 are also charted. Each was arrived at as follows.

TABLE 13

The Average Temperature (F°) at Provincetown, Cape Cod, Massachusetts, Week-by-Week for the Periods of Obelia Collections from 1933 to 1939 Inclusive

Period	1933	1934	1935	1936	1937	1938	1939
April							
1	——	——	——	——	——	——	42.0
2	——	——	——	——	——	——	43.3
3	——	——	——	——	——	51.2	48.3
4	——	——	——	——	——	49.5	46.7
May							
1	52.7	——	53.4	55.6	54.1	52.1	48.6
2	51.6	——	55.9	53.8	54.4	52.2	56.3
3	58.4	61.6	56.0	56.5	55.5	54.4	52.2
4	59.8	58.5	60.7	60.5	59.7	54.1	55.8
June							
1	61.0	61.3	60.9	63.9	63.4	58.6	61.9
2	65.5	65.8	64.6	63.2	64.8	60.8	63.1
3	61.4	67.5	61.6	66.1	64.7	61.0	66.2
4	71.4	70.5	71.2	64.3	63.2	67.0	62.6
July							
1	62.5	76.1	71.6	69.1	71.4	62.7	68.6
2	60.0	68.4	75.8	72.5	70.4	66.3	68.7
3	69.6	74.8	75.1	69.8	70.7	67.6	64.7
4	68.9	73.0	74.1	71.3	72.2	70.3	69.3
August							
1	67.9	72.5	73.0	70.6	74.1	72.5	71.8
2	67.9	68.2	74.1	72.0	75.9	66.8	75.6
3	68.9	68.4	74.4	67.5	75.0	71.0	72.2
4	70.1	64.6	68.9	68.2	65.9	68.4	69.4
September							
1	67.8	68.8	66.7	64.6	69.6	64.0	66.5
2	61.1	66.1	63.9	64.9	63.6	60.5	63.0
3	61.8	——	——	——	61.7	——	——
4	58.8	——	——	——	——	——	——

The highest and lowest temperatures for each 12-hour period beginning at midnight for each day of experiment were taken from the continuous record given by a Tycos Thermograph kept in sheltered shade. This gave four readings for each day—two high and two low. The sum of these for all the days of experiment done in a given week was taken, and the whole divided by four times the days of experiment for the week. A specific example will illuminate. In 1936 the first "week" of June had six days of experiment. The number of readings was four per day for six days, or 24. The sum was 1,534.0 degrees. The average temperature was 1,534.0 divided by 24 or 63.9°. And so on similarly. In this manner temperature is seriated with colony composition.

The charts show that colony composition is subject to season change. This is chiefly compounded of shifts in percentage of buds and complete hydranths. A progressive decrease in percentage of cylinders is a minor contributor. None of the other elements, viz., cones, seniles, empties, and brokens exhibit changes which can be classed as seasonal.

Bud percentage starts high in April and/or May and drops sharply during May and/or June to a summer characteristic low level. There is evidence of some increase sometime in July or August over the previous low and a subsequent drop from the preceding high; but the timing and extent of these shifts are too inconsistent for anything but mention of the possibility. The overall picture is one of season shift from high level in springtime to low level in summer. Colonies collected in spring have more buds than those collected in summer. This means that more buds are produced in spring than in summer. Hence that the growth activity of initiation is expressed to greater degree in spring than in summer.

The complete hydranths on the other hand start low in April and/or May and increase during June, July, and August. They may drop off slightly in September. The overall picture is one of a season shift from low value in springtime to high values in summer. Colonies collected in the spring have fewer complete hydranths than those collected in the summer. It is through complete hydranths that nourishment for the colonies is obtained. This season shift in their percentage contribution to colony composition means that the maintenance capacity of summer colonies is greater than that of springtime.

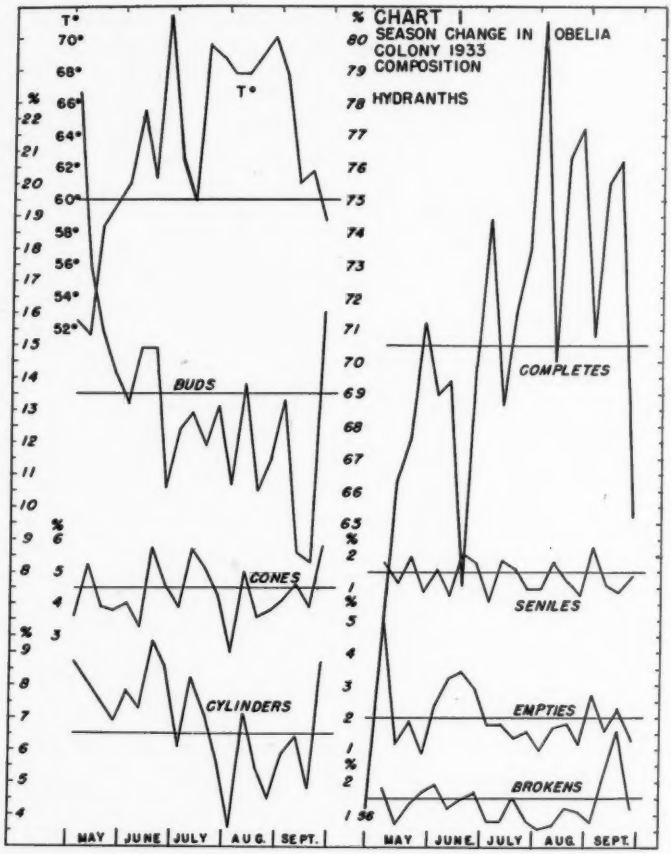
CHART I
SEASON CHANGE IN OBELIA COLONY 1933
COMPOSITION
HYDRANTHS
T°
BUDS
CONES
CYLINDERS
COMPLETES
SENILES
EMPTIES
BROKENS
MAY
JUNE
JULY
AUG.
SEPT.

CHART 1

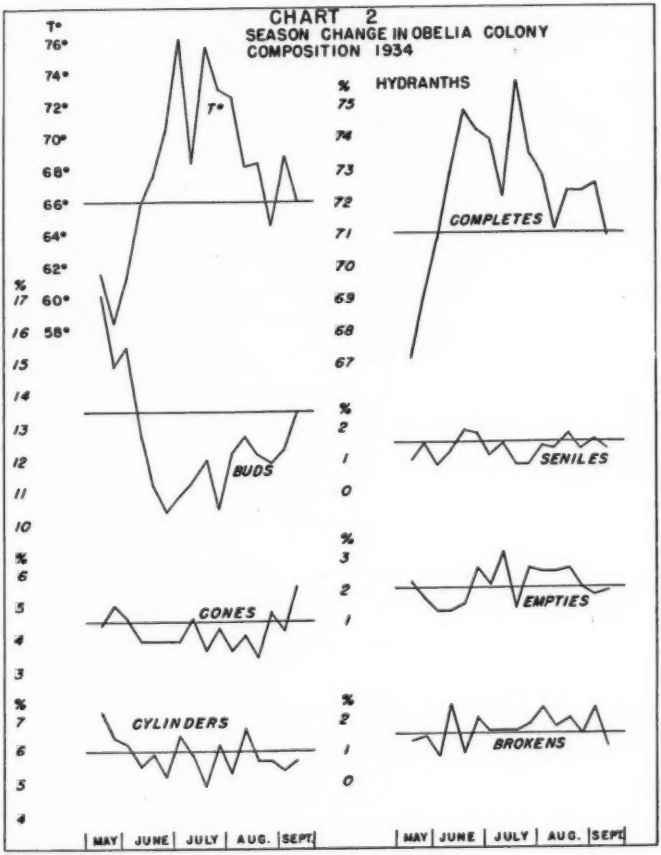
CHART 2
SEASON CHANGE IN OBELIA COLONY
COMPOSITION 1934
T°
76°
74°
72°
70°
68°
66°
64°
62°
60°
58°
T°
%
17
16
15
14
13
12
11
10
BUDS
%
6
5
4
3
CONES
%
7
6
5
4
CYLINDERS
% HYDRANTHS
75
74
73
72
71
70
69
68
67
COMPLETES
%
2
1
0
SENILES
%
3
2
1
EMPTIES
%
2
1
0
BROKENS
MAY JUNE JULY AUG. SEPT
MAY JUNE JULY AUG. SEPT

CHART 2

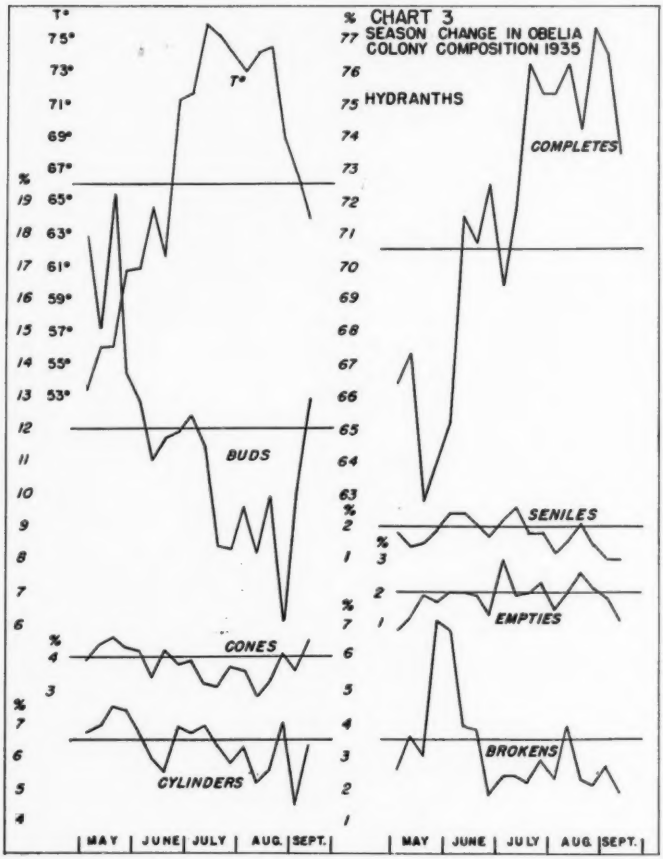
CHART 3
SEASON CHANGE IN OBELIA
COLONY COMPOSITION 1935
T°
HYDRANTHS
BUDS
CONES
CYLINDERS
COMPLETES
SENILES
EMPTIES
BROKENS
MAY
JUNE
JULY
AUG.
SEPT.

CHART 3

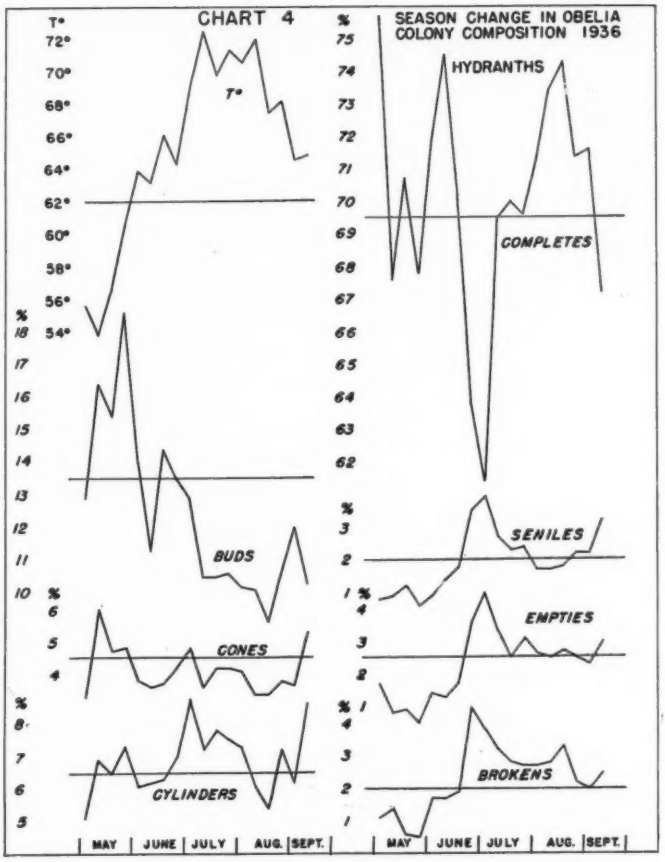
CHART 4
SEASON CHANGE IN OBELIA COLONY COMPOSITION 1936
T°
72°
70°
68°
66°
64°
62°
60°
58°
56°
54°
T°
%
18
17
16
15
14
13
12
11
10
BUDS
%
6
5
4
GONES
%
8
7
6
5
CYLINDERS
%
75
74
73
72
71
70
69
68
67
66
65
64
63
62
HYDRANTHS
COMPLETES
%
3
2
1
SENILES
%
4
3
2
EMPTIES
%
4
3
2
1
BROKENS
MAY
JUNE
JULY
AUG.
SEPT.
MAY
JUNE
JULY
AUG.
SEPT.

CHART 4

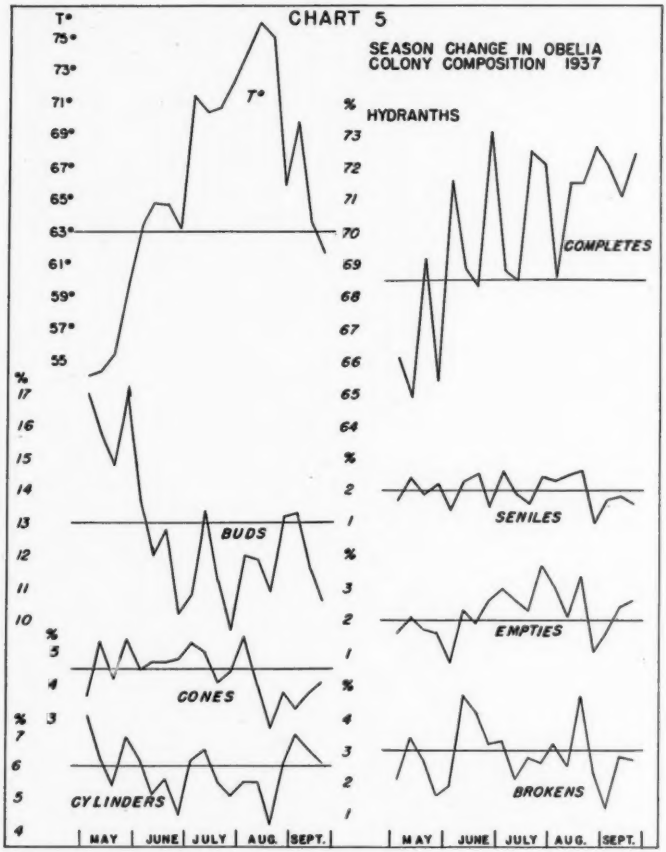
CHART 5
SEASON CHANGE IN OBELIA COLONY COMPOSITION 1937
T°
75°
73°
71°
69°
67°
65°
63°
61°
59°
57°
55
T°
%
HYDRANTHS
73
72
71
70
69
68
67
66
65
64
COMPLETES
%
17
16
15
14
13
12
11
10
BUDS
%
2
1
SENILES
%
3
2
1
EMPTIES
%
5
4
3
CONES
%
4
3
2
1
BROKENS
%
7
6
5
4
CYLINDERS
MAY
JUNE
JULY
AUG.
SEPT.
MAY
JUNE
JULY
AUG.
SEPT.

CHART 5

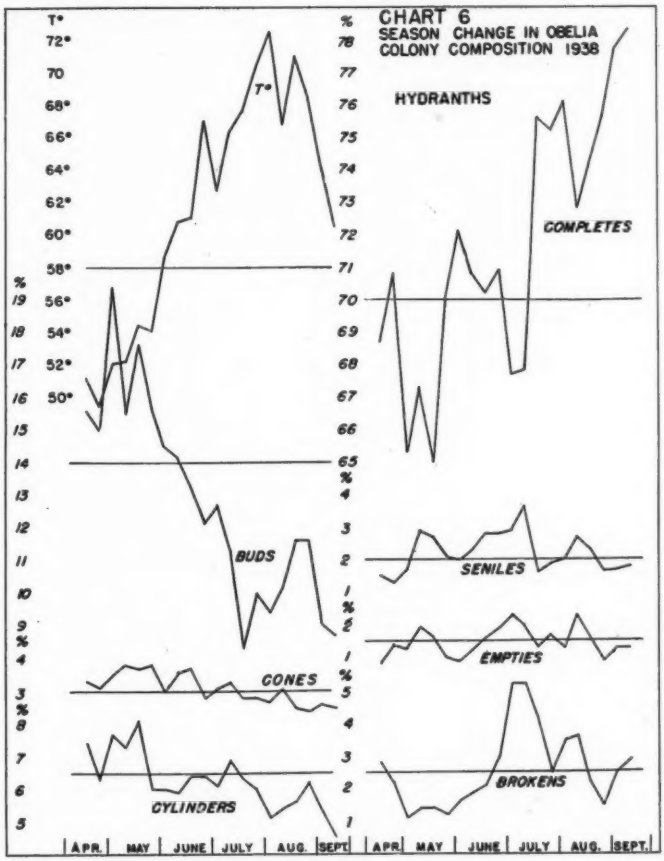
CHART 6
SEASON CHANGE IN OBELIA
COLONY COMPOSITION 1938
T°
72°
70
68°
66°
64°
62°
60°
58°
56°
54°
52°
50°
%
78
77
76
75
74
73
72
71
70
69
68
67
66
65
HYDRANTHS
COMPLETES
T°
BUDS
CONES
CYLINDERS
SENILES
EMPTIES
BROKENS
APR.
MAY
JUNE
JULY
AUG.
SEPT.

CHART 6

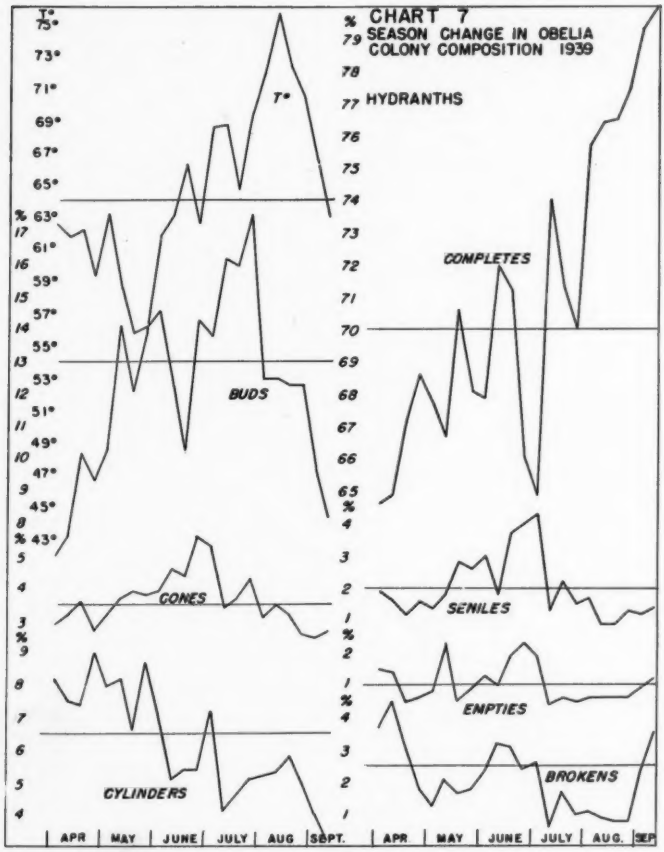
CHART 7
SEASON CHANGE IN OBELIA
COLONY COMPOSITION 1939
HYDRANTHS
T°
BUDS
CONES
CYLINDERS
COMPLETES
SENILES
EMPTIES
BROKENS
APR
MAY
JUNE
JULY
AUG
SEPT.

CHART 7

These season-associated shifts in buds and completes don't just happen. They are the result of factors operating on or within the colonies.

Operating on the colonies are the exogenous season-determined changes in temperature, illumination, salinity, pH, and plankton.

Operating within the colonies are endogenous factors based in the activity inter-relationships of the different structural states.

As pointed out elsewhere each structure is the product of a dominantly definitive activity. Each evolves from an antecedent structure, and is itself the genitor of a succedent. The colony lives as a whole, not as a collection of unrelated units (Hammett, 1942, 1943). Colony composition is thus the resultant of a balanced inter-play between the several activities. Such being the case it is clear that this interdependence must be taken into account in any study of composition change.

Now the charts show that—

1. The course of season change in buds is essentially opposite to that of the complete hydranths.

2. The course of season change in buds is roughly opposite to that of temperature. And—

3. The course of season change in complete hydranths is roughly like that of temperature.[3]

These graphic correlations suggest that season change in buds and completes is temperature determined.

1. *Buds: Temperature*

But bud production drops as temperature rises. If the bud drop is interpreted as due to temperature rise one would have to postulate a critical high inhibitory temperature. Such is possible for any physiological activity. But that this is the case here is doubtful. For the following reasons.

Only the buds experience the sharp springtime decrease. No other hydranth structure exhibits this change. It could be expected that other structures would be retarded if temperature rise is here critically inhibitive. On the other hand each colony component is subtended by its own set of chemical reactions. A temperature critical

[3]Temperature is here Fahrenheit and atmospheric. But its season change is like that of waters in similar latitudes (Sverdrup, Johnson, and Fleming, 1942).

for one might not be critical for another. So, while the specificity of bud decline casts doubt on the critical temperature hypothesis, it fails to dispose of the matter.

Table 14 gives selected temperature and bud data for beginning and terminal periods of the early sharp drop in bud production. Also bud percentages and temperatures at later higher temperatures. The figures are transcripts from Tables 5 to 13 (q.v.). Increases in temperature; and percentage changes in buds from one stated period to the next are set down; singly and as averages.

The data show that the greater part of bud springtime decrease occurs as temperature rises within the 54- to 63-degree range. It is a little unusual for a rise at such a low level to be critically inhibitory of growth in this latitude. This strengthens doubt in the critical temperature hypothesis, but does not dispose of it.

If the drop in bud production is due to a critical rise in temperature, it could be expected that further rise would be still more inhibitory. But bud production at 71.6° may be no less than at 63.3°. In fact buds increase with late temperature rise, in four of the seven years, and decrease in but two. And the decreases are much less during a rise from 60° to 71° than during a rise from 51° to 60°. The figures are averages. The picture as a whole allows the conclusion that the springtime drop in bud production is not a result of temperature increase to a critically inhibitory level.

This kills the idea that the course of season change in buds is temperature determined. It will do no harm to bury the corpse and get it out of sight. Such is done by comparing the week-to-week changes in direction of bud percentage with those of temperature. For if temperature is a major factor it could be expected there would be correlation betwen bud change and temperature change.

Table 15 gives the frequency with which direction of bud change was the same as that of temperature; the frequency with which it was opposite; and the number of times comparison could not be made because one or the other failed to change.

Interest is in consistency of concordance. So any change is taken as indicative and so recorded in the "Ind." lines. Some may cavil at inclusion of slight differences. So a series is given where such are put in the "no-comparison" bracket. This distribution is put in the "Sig." lines. The trends are essentially the same in both classifications.

TABLE 14

COMPARING SELECTED SEASON CHANGES IN BUD PRODUCTION WITH THE CONCOMITANT SEASON INCREASES IN TEMPERATURE (FAHRENHEIT)

A.D.	Period A	B	C	Temperature A	B	C	B-A	C-B	Bud production A	B	C	B/A	C/B
'33	MY1:	JN1:	AG2	52.7	61.0	67.9	8.3	6.9	22.8	13.2	*13.8*	—42.1	+ *4.5*
'34	MY3:	JN4:	JL3	61.6	70.5	74.8	8.9	4.3	17.1	10.4	*12.0*	—39.2	+*15.3*
'35	MY1:	JN2:	JL2	53.4	64.6	75.8	11.2	11.2	17.9	11.0	*11.4*	—38.5	+ *3.6*
'36	MY2:	JN2:	JL2	53.8	63.2	72.5	9.4	9.3	16.4	11.3	*10.5*	—31.1	— *7.0*
'37	MY1:	JN2:	JL2	54.1	64.8	70.4	10.7	5.6	17.0	12.0	*13.4*	—29.4	+*11.7*
'38	MY1:	JN4:	AG3	52.1	67.0	71.0	14.9	4.0	19.4	12.1	*11.6*	—37.6	— *4.1*
'39	MY1:	MY3:	JL1	48.6	52.2	68.6	3.6	16.4	17.6	13.9	*13.8*	—21.0	— *0.7*
	Averages			53.8	63.3	71.6	9.6	8.3	18.3	12.0	*12.4*	—34.1	+ 3.3

A.D.—Year; MY—May; JN—June; JL—July; AG—August.

TABLE 15

CONCORDANCE OF WEEK-TO-WEEK CHANGE OF: A. BUD PER CENT WITH THAT OF T°; B. COMPLETE PER CENT WITH THAT OF T°; C. BUD PER CENT WITH THAT OF COMPLETE PER CENT

	%			Totals			April			May			June			July			August			Sept		
	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N
A. Buds on temperature																								
126	43	50	7	54	63	9	3	1	0	10	10	1	8	19	1	11	14	3	13	11	4	9	8	0
Weeks	36	44	21	45	55	26	3	1	0	9	8	4	8	16	4	8	14	6	9	9	10	8	7	2
37	41	51	8	15	19	3	2	0	0	3	4	0	1	6	0	3	4	0	3	3	1	3	3	1
Mth-Yrs	38	54	8	14	20	3	2	0	0	3	3	1	1	6	0	2	4	1	3	4	0	3	3	1
23	44	52	4	10	12	1	2	1	0	2	2	0	0	4	0	2	2	0	2	2	0	2	1	1
Periods	39	52	9	9	12	2	2	1	0	2	2	0	0	4	0	2	2	0	2	2	0	1	1	2
B. Completes on Temperature																								
126	50	45	5	63	57	6	2	2	0	8	12	1	18	10	0	15	12	1	12	12	4	8	9	0
Weeks	43	37	20	54	47	25	1	2	1	7	11	3	16	8	4	12	9	7	11	10	7	7	7	3
37	43	46	11	16	17	4	1	1	0	3	4	0	3	4	0	3	1	3	3	3	1	3	4	0
Mth-Yrs	49	32	19	18	12	7	0	1	1	3	3	1	5	1	1	3	2	2	4	2	1	3	3	1
23	48	30	22	11	7	5	2	0	1	1	2	1	4	0	0	2	2	0	1	1	2	1	2	1
Periods	52	22	26	12	5	6	1	1	1	2	1	1	4	0	0	2	1	1	2	1	1	1	1	2
C. Buds on completes																								
126	23	71	6	29	89	8	1	3	0	2	19	0	10	17	1	9	18	1	3	19	6	4	13	0
Weeks	21	61	18	26	77	23	1	2	1	2	18	1	10	16	2	8	13	7	3	16	9	2	12	3
37	8	78	14	3	29	5	0	2	0	0	7	0	1	4	2	1	4	2	0	7	0	1	5	1
Mth-Yrs	11	70	19	4	26	7	0	1	1	0	7	0	1	4	2	2	3	2	0	6	1	1	5	1
23	4	92	4	1	21	1	1	2	0	0	4	0	0	4	0	0	4	0	0	3	1	0	4	0
Periods	4	78	17	1	18	4	1	1	1	0	4	0	0	4	0	0	2	2	0	3	1	0	4	0

Top line of all pairs significant: Bottom line indicative. S—Same Direction; O—Opposite Direction; N—No comparison.

In order to equilibrate for possible weighting, the data are treated in four different ways. The week-to-week change is basic to all.

There are 126 separate opportunities for buds and temperature to change from a preceding status. The first set of figures gives the frequency with which the direction of bud change on these 126 occasions was the same, opposite, or not comparable with that of temperature. The data are combined into one distribution for the picture as a whole, and separated into the distribution extant in each month for the season progress picture.

The totals show that buds follow temperature in 43 per cent of cases, run opposite in 50. In three of the six months there is no correlation, and the negative correlation in two is partly neutralized by the positive in one. This series gives no evidence that bud change is consistently correlated with temperature change, either seasonally or as a whole.

The foregoing is compounded of records for separate years and separate periods. It could happen that the distribution for a given month is weighted by the trend of a single year or period.

The data for May, June, July, August, and September each comprise records for seven years; those of April for two. If we record for each year separately in each month the frequency with which its periods exhibit sameness, oppositeness, and no-comparison, we get distributions which show whether the trend in each of the 37 month-years is towards sameness, oppositeness, or no-comparison. The second series of figures in Table 15 gives the distribution of these trends; both as a whole and by months.

The totals show that buds follow temperature in 41 per cent of cases, and run opposite in 51. In four of the six months there is essentially no correlation, and the negative correlation in one is balanced by the positive in the remaining. This combination is consistent with the preceding, and shows there is no weighting by the data of any one year.

The data for May, June, July, August, and September each comprise records for four separate periods; those of April for three. If we record for each period separately in each month, the frequency with which sameness, oppositeness, and no-comparison is exhibited by the component years, we get distributions which show whether the trend in each of the 23 month-periods is towards sameness, oppo-

siteness, or no-comparison. The third series of figures in Table 15 gives the distribution on this basis, both as a whole and by months.

The totals show that buds follow temperature in 44 per cent of cases, and run opposite in 52. Four of the six months show no correlation, and the negative correlation in one tends to be balanced by the positive trend in the remaining. This combination is consistent with the preceding, and shows there is no weighting by the data of any one period.

Finally it could happen that the distribution of the first series totals is weighted by the trend of a single year. To equilibrate for this we have gotten the frequency of sameness, oppositeness, and no-comparison for each of the seven years separately by combining the distributions in all periods of all months of record for each year singly. In order to save complication the results are not set down. It is sufficient to state that there is essentially no bud-temperature correlation in four of the seven distributions so obtained, while the negative correlation in two is partially neutralized by the positive in one. This combination is consistent with the preceding, and shows there is no weighting of the first series totals by the data of a single year.

This also indicates the need for collecting data over several years. If records had been had only for 1933 and 1935 it might have been concluded that buds and temperature are negatively correlated. If 1939 had been the only year of record the opposite decision might have been made. But having these and those for 1934, 1936, 1937, and 1938 where no correlation is found, it is evident there is no overall relation of annual occurrence during the months of record.

These data then give but one conclusion, which is that *the course of season change in buds is not determined by the course of season change in temperature.*

Let no one read into this that temperature is at no time or in no way a factor in budding. That would be absurd. When winter-dormant colonies are brought from their natural habitat into the laboratory they start budding in short order as they warm up. Everybody knows there is a critical low temperature below which growth is inhibited. And of course initiation in Obelia as in all other organisms is released from inhibition by a rise in temperature above the critical low level. The positive correlation between buds and temperature in April is

expression of this. But what happens after this is not a matter of temperature control.

With temperature eliminated we turn attention to other season-associated factors of possible significance to bud production.

2. *Buds: Illumination*

Our data are inadequate to show whether or not season change in light duration has any influence on season change in Obelia budding. It is true that buds decrease as the days lengthen (April to late June) and may tend to increase as they shorten (July-August). And it is true that colonies collected in the morning in April and September have more buds than those collected in the afternoon. But to conclude from such meagre data either that light is retardative of budding, or that day length is important thereto would be to let imagination run away with good sense.

3. *Buds: pH*

The slow season fall in pH could be of influence. But record is had for only one year and none for the early weeks of major bud decline. Attempt at integration would be futile.

4. *Buds: Plankton*

While season rise and/or change in plankton might be a factor in the observed changes in buds, explanation is elusive and none will be attempted.

5. *Buds: Salinity*

Sverdrup, Johnson, and Fleming (1942) give a charting on page 162 of season change in surface salinity in the North Atlantic Ocean between latitudes 18°N and 42°N. Our Obelia were collected right on 42°N and the organism lives in surface waters. These authors report that euryhaline organisms may be affected by salinity since those living in reduced salinities frequently have smaller maximum size than those living in more saline waters.

The chart shows a high salinity in March and May, a drop to a low in June, a rise from June to July, with the August value dropping, but still above the June level, and a continuing drop from September to November.

The season course of bud change matches that of salinity to remarkable degree. Buds are high in April and May; they drop greatly from May to and in June; they pretty consistently exhibit some rise from the preceding low sometime in July and/or August; and they show a drop from the preceding high sometime during August and/or September.

Salinity in shore areas is factored by rainfall, drainage, and evaporation. The ups and downs of buds and salinity coincide with the year-by-year impression that springtime thaws and rains dilute the shallow waters of our Bay; that the long periods of hot dry weather of the summer raise salinity by evaporation; and that dilution is again effected by the late season rains.

The bud-salinity-weather concordance in season change seems almost too true to be good. But when it is taken together with the records of reproduction stimulation and retardation by changing salinity (Flattely and Walton, 1922; d'Arcy Thompson, 1942) it looks as if the course of season change in salinity might be a significant factor in the course of season change of buds.

The year-by-year irregularity in time of bud change is consistent with the idea. For if salinity is a major factor, and if its season changes are sequalae of rainfall and evaporation, then its season influence would arrive in different weeks in different years, since the rain and hot dry days arrive in different weeks in different years.

All this is very suggestive. But detailed year-by-year records of local salinity are lacking. So confirmatory or eliminatory analysis such as was made with temperature is impossible. All it is proper to do here is point out the implication.

If the positive correlation between buds and salinity is real it could be inferred that the high bud production of early spring, and the late summer rises from preceding lows, are due in part to a fostering of bud production by a critical high level of salinity, while the subsequent declines in bud production are in part consequences of its concurrent decreases.

6. *Buds: Endogenous Factor*

It is poor science which fails to consider alternatives. And there is another factor which may contribute to season-associated highs in bud percentage.

Buds derive not only from anlagen which develop within the hydro-

caulal wall, but also from reconstituted cells of the distal ends of pedicels wherefrom previously existing hydranths have disappeared. So, the buds found in any collection of colonies are not all new additions to colony population. Many may be beginning replacements by recurrent growth of hydranths which have completed their life-cycle and passed away in the natural course of events. And some may be replacements by regeneration of hydranths lost by injury.

Our observations (without record) make it seem possible that the rigors of winter ice and storms cause increase in the *zu grunde gehen* of functioning hydranths, and the breakage of their pedicels. Combining this with the fact that the chemical processes of replacement are inhibited by low winter temperature, it could be that colonies at end of winter have more bare and broken pedicels than in spring or summer, when damage is less and followed by growth progression to more advanced states since replacement is uninhibited.

If this be so it is obvious that colonies at end of winter have more foci from which buds may spring than colonies in spring or summer. Hence the release from inhibition of processes concerned in replacement by the waning winter rise in temperature above a critical level, would automatically result in higher bud percentages in early spring colonies than in those of late spring and summer of assumedly fewer foci for bud production.

In the same direction is the fact that the July-August rise in bud percentage is generally preceded by an increase in foci for bud production by recurrent growth and regeneration. It can be found from Tables 5 to 11 that in five of the seven years the sum of the senile, empty, and broken percentages increases the week before the rise in buds. The late-summer increases in bud percentage may therefore also be simply a consequence of the immediately antecedent increase in foci and hence potentiality for bud production.

These correlations suggest that salinity is not necessarily the determinant of season change in budding.

On the other hand the gonangial data tell another story.

An index of season change in colony gonangia production is had in the number of gonangia per 10 colonies in each successive week. The combined data for the six years from 1934 to 1939 inclusive are given in a single curve in Chart 8.

In our experience, gonangia do not customarily exhibit recurrent or

regenerative growth. They do not ordinarily (like the hydranths) regrow from pedicels on which members have previously lived, grown, and passed away in the normal course of events, nor from pedicels from which members have been lost by injury. In fact from such, a hydranth might be expected as likely as not, if Hauschka's results may be taken as guide (1944). Such being the case, winter battering cannot be expected to increase the number of foci for gonangial production.

The curve is pretty evidence supporting this deduction. The gonangia start low in early April and then sharply increase in late April. This shows that winter damage is not the factor responsible for the springtime increase in productive foci. Else the earlier colonies would have had more gonangia and the later fewer.

What then is the season-associated factor responsible for the great rise in gonangia production of early spring?

Chart 8 also gives the course of season change in gonangial bud percentage (number of buds per 100 gonangia), with the data of the six years combined into a single curve.

This shows that gonangial buds follow a course which is quite like that of hydranth buds. There is the same high productivity of early spring; the same drop to a June low; the same suggestion of a mid- or late-summer rise; and the same decline thereafter.

The course, then, like that of the hydranths, is matched in essential respects by that of salinity. And here there isn't the probability that the early spring and mid-summer highs are contributed to by increases in foci resulting from antecedent regression and/or destruction of previously existing members.

Since an antecedent passing away or pre-existing structures is apparently not necessary to the season highs in gonangial bud production, it follows that it also may not be necessary to the concomitant season highs in hydranth bud production.

This brings us right back to where we started from, namely to salinity. And the gonangial data, by eliminating a stumbling block encountered in the hydranth data, renew the possibility that the course of season change in bud production is factored in some degree by the course of season change in salinity.

Further speculation would be unprofitable. The matter can only be decided by experiment and more observations. So now to the

completes as the only other component of hydranth population consistently showing marked season changes.

7. *Complete Hydranths: Temperature*

Charts 1 to 7 show that the course of season change in contribution of complete hydranths to colony composition is grossly similar to the course of season change in temperature. This suggests that temperature may be a determinant. If this be true it could be expected that the week-to-week direction of change in completes would correspond with that of temperature.

Section B of Table 15 shows that such relation does not exist. While there is sameness in 50 per cent of the 126 changes there is oppositeness in 45. And in three of the six months there is no correlation, while the two which show positive are partially balanced by the one where negative is present.

Similarly, in the segregation by month-years, sameness is present in 43 per cent of the 37 comparisons, and oppositeness in 46. While in five of the six months no correlation is found, and positive exists in but one.

It is only in the totals of the period segregation that sameness is dominant. Here 48 per cent of the 23 comparisons show concordance, while non-concordance is found in but 30. On the other hand positive correlation is exhibited in but two of the six months, and two show no correlation, whereas two may have a hint of negative.

Furthermore, while sameness may dominate in three of the seven years, oppositeness also rules in three, and no correlation is found in one.

Finally while complete percentage increases with rise in temperature in 59 per cent of the 70 instances of percentage rise, it increases in the face of falling temperature in 40. And though it falls with falling temperature in 42 per cent of the 53 instances of percentage drop, it falls in the face of rising temperature in 55.

These segregations give no evidence of consistency of relation. This outcome was not expected. For knowledge that processes are usually advanced by temperature rise (within limits) and retarded by its fall, combined with the rough positive correlation of season trend in the two variables, would seem to indicate factorial relation. The fact that the break-down analysis, which was made just as a check, is not

supportive, raises the question as to whether its results may not be skewed by some extraneous factors. Two possibilities come to mind.

It is conceivable there is some lag or some discrepancy between the weekly average atmospheric temperature and that of the surface water. The which might act to give correlations where none existed in actuality; and to give no correlations where such were present. But it is our experience that the shallow surface water of our shores is warm when the day is warm, and cold when the day is cold. Moreover our tide comes in slowly over a great expanse of sand-flats; its maximum depth over the colonies during the months of collection is rarely more than two or three feet; and the period during which the colonies are most deeply submerged is about four hours out of the 24. It seems improbable that the direction of change in surface water temperature from week to week differs greatly from that of the atmosphere with sufficient frequency to skew the data into invalidity.

It has been repeatedly observed that regression of complete hydranths to the senile state is markedly enhanced on hot days in colonies in laboratory culture. A rise in temperature above a certain level may determine a decrease in percentage contribution of the completes to colony composition. Occasion transitory intrusion of such regression-producing high temperature during July and August is to be expected.

So it is conceivable that positive correlations of temperature rise with rise in percentage of completes (within the non-regression-stimulating range) which might serve as evidence of factorial influence, could be cancelled out by the presence of negative correlations derived from temperature rise to the regression-stimulating (percentage decreasing) level. The end-result would be a black-out of evidence of consistency in complete hydranth-temperature relation.

Now it can be shown that in May and June the percentage of completes falls in the face of rising temperature almost as often as it rises therewith. But, although a goodly rise in temperature occurs during these months, it does not, in our experience, reach the regression-enhancing level. So the negative correlations are not attributable to temperature rise. Since even in the non-regression-enhancing temperature range there is no consistency in complete hydranth-temperature relation—the positive and negative correlations being essentially balanced—it looks as if the segregation data are reliable indices of

the true status. Such being the case it is apparent that *some factor other than temperature is directly responsible for the course of season change in complete hydranths.*

Let no one take from this any idea that temperature is without influence on the completes. That would be a mistake. All that is here established is that the *course* of season change peculiar to the completes is not directly determined by the course of season change in temperature. Only this and nothing more.

It is noteworthy that the increasing contribution of completes to colony composition is interrupted by a sharp drop in the first or second week of July. This is accompanied by an increase in seniles and/or empties. It coincides in most years with the new moon, spring tides, and unusual warmth of surface water. This has been found as high as 86°F. The phenomenon is an example of how temperature rise to critically high level may determine decrease in completes by enhancement of regression.

This mechanism then is undoubtedly one of the factors contributing to colony destruction noted by McDougall (1943) and Grave (1933), for there is correlation between the northward march of warm weather and colony disappearance and disintegration. This happens by mid-June at Beaufort, at the end of June at Woods Hole, and by mid-July here at North Truro to colonies growing in exposed locations.

On the other hand direct action of high temperature is not necessarily paramount. In our experience colonies survive such highs when living in the caves and grottoes of our breakwater. Here disintegration is delayed until mid-September and seems more to be due to invasion by predatory protozoa than to anything else. As established by repeated observations under the microscope in the laboratory. Season rise in temperature is undoubtedly a direct and/or indirect factor in the season increase in these destructive parasites. But the progressive downfall of cave-living Obelia is largely due to these; not to direct stimulation of regression by high temperature.

Furthermore if one observes colonies growing exposed on rocks and piling it is evident that their destruction is largely a matter of desiccation. Rocks and piles dry out more and sooner on the long warm days of early summer than on the cool short days of early spring. The colonies get dry as the water left thereon by the receding tide evaporates. And glistening tiny crystals of salt are left thereon.

The exosmosis possibly induced by this abnormally high salt concentration on colony surface, plus the desiccating effect of wind and sun are responsible for the early-season disappearance of colonies, not high temperature as such.

This is no attempt to minimize the importance of temperature. What we are trying to do is to track down where it comes into the picture in season change in Obelia colony composition. If the data yield no evidence that it is directly concerned in the course of season change in buds and completes, it is the facts that yield the conclusion; not the conclusion the facts.

The analysis brings out two important side-issues. The first is that critical examination of data underlying graphic or any other apparent correlations should be made before jumping to conclusion of causal relations. And the second is that valid approximation to knowledge of season differentials can only be had when habitat is taken into account and records made of numbers of organisms at different stages of development.

8. *Complete Hydranths: Illumination*

The completes increase as the days get longer. They do not decrease as the days get shorter. They contribute as much to colony composition in colonies collected in the morning after long hours in the dark of night, as they do to those collected in the afternoon after hours of exposure to the light of day. The data yield no evidence that light is a factor in season change in completes.

9. *Complete Hydranths: Salinity*

The data give no hint as to whether or not salinity is of influence on the course of season change in completes. Other than the possibility noted above in respect to colonies growing exposed on rocks and pilings. But this is not pertinent to the material of the present study.

10. *Complete Hydranths: pH*

Completes derive from cylinders. And cylinders are high in early spring. So the springtime rise in completes is contributed to by the high springtime level of the cylinders. Cylinder production is fostered by high pH (Hammett and Rivard, 1940). And pH is higher

in spring than thereafter. So the higher springtime pH may be in part a factor in the higher springtime level of cylinders. If this be so the higher springtime pH may contribute to the springtime rise in completes through fostering production of their antecedent cylinders. In this tenuous way, and this way only, can season change in completes be tied in with season change in pH from these data.

11. *Complete Hydranths: Plankton*

There is no record of local plankton save impression of season increase. But complete hydranths pass on to seniles in laboratory culture of meagre plankton with greater frequency than in their natural habitat where plankton is abundant. This suggests that plankton level may factor persistence of complete hydranths. Conceivably the colony adjusts itself to low nutrition by reducing the number of feeding animals. Conversely season increase in food supply could favor persistence and hence increase in percentage of completes. This is pure guess and should be taken as such.

12. *Complete Hydranths: Endogenous Factors*

Charts 1 to 7 show the course of season change in completes is roughly opposite that of the buds. This suggests a relationship. Such is verified by the fact that direction of change in completes from week to week is consistently correlated with that of the buds.

Section C of Table 15 shows that the completes increase and decrease oppositely to the buds in 71 per cent of the 126 changes and follow the buds in but 23. This negative correlation occurs in each of the six months separately.

Similarly, the segregation by month-years shows oppositeness in 78 per cent of the 37 comparisons, sameness in but 8. And the negative correlation is present in each month.

In the period arrangement oppositeness occurs in 92 per cent of the 23 comparisons, sameness in but 4. And negative correlation rules in each month.

Finally, negative correlation dominates, and not weakly, in each of the seven years of record, when the data are combined into a single distribution for the single year.

The ubiquity and consistency of the negative correlation between completes and buds with respect to direction of change from week to

week with advancing season, when taken in conjunction with the fact that the completes increase and the buds decrease on season, and the fact that temperature, light, salinity, pH, and plankton cannot be satisfactorily integrated therewith in factorial relation, strongly suggest that the course of season change in completes is in large measure endogenously determined. Sustaining evidence is not lacking.

Complete hydranths derive from growth of buds, cones, and cylinders. They pass on by regression and catabolism to senile animals and empty hydrothecae. When growth activity is greater than regression, the percentage of completes increases; when regression activity is greater than growth, the percentage decreases.

But a third endogenous factor comes in to complicate matters. This is the relative duration of the hydranths in their several structural states. Anlagen go on to buds, buds to cones, cones to cylinders, cylinders to completes, all in a few short hours. And the same is true of senile progression to empty hydrothecae and the formation of new buds therein by recurrent growth. But the complete hydranths may stay as completes for days on end.

Evidence of this is had not only from the cinematic record noted in the preceding paper (Hammett, 1943), but also from the fact that the number of completes under usual conditions is always much greater than the sum of all the other states combined; the fact that progression from the complete state during the 24 hours of laboratory culture is always less than the progression from any other state (Hammett, lit. 1943); and the fact shown in Tables 5 to 11, that the sum of the buds, cones, and cylinders decreases as season advances, while that of the seniles, empties, and brokens tends to stay the same.

The number, and the percentage of the completes is thus a matter of the quality of persistence of this colony element, as well as of the balance between the oppositely acting activities of growth and regression.

This quality of persistence is grounded in chemical processes specific and peculiar to the completes. It, together with the balance between growth and regression, is endogenous in nature.

Picturization is simple. The tables show that buds are continuously produced by the growth activity of initiation from April to September. These do not stay as buds, but continuously go on by proliferation to cones. The cones do not stay as cones, but continuously go on by

differentiation to cylinders. And the cylinders do not stay as cylinders, but continuously go on by organization to completes. The number of completes resulting from this continuous growth progression is greater than the number which goes on by regression and catabolism to seniles and empties; because of the quality of persistence indigenous to the completes. So the number, and the percentage of completes increases as season advances. And the large springtime increase is the natural consequence of the large springtime production of buds.

Hence, since season change in completes is a resultant of season change in the endogenous balance between the activities of growth, regression, and persistence, it is logical to conclude that the course of *season change in completes is in large measure endogenously determined.*

While this seems fair enough the matter of season change in buds is troublesome. Why is it that bud production drops so precipitously after the first high activity of early spring? Has the concomitant sharp rise in completes anything to do with the phenomenon? It is convenient enough to invoke salinity; but isn't it possible that this is of minor import, and that the course of season change in buds —like that of the completes—is also in large measure a matter of endogenous determination? The data do indicate that once the colonies acquire a certain quota of complete feeding hydranths, bud production is essentially reduced to that required for maintenance of the quota in the face of the wear and tear of existence. With some slight excess for colony expansion, but not much.

It could be that there is an inhibiting agent for anlagen constitution, and that this is produced by chemical processes peculiar to the completes. Analogically such is possible. Increase in number of completes would mean increased production of this hypothetical inhibitor, and increased concentration thereof in the hydroplasm. The result would be a cut in anlagen production, and fewer buds. Buds would decrease as completes increase, and increase as completes decrease. Which is what happens. It is not impossible that some such mechanism is concerned in the fine equilibrium of buds vs. completes in this concinnate assembly.

So the springtime drop in buds may be in part due to decrease in salinity, in part to decrease in untenanted pedicels, and in part to

increased concentration of an inhibitant to anlagen constitution by hydrocaulal cells. The conclusion is that the course of season change in buds may be in large measure endogenously determined.

Now to clean up some odds and ends.

13. *Cylinders*

The contribution of this element to colony composition decreases as season advances in six of the seven years of record. pH also decreases. Experiment shows that cylinder production from cones is favored by high alkalinity (Hammett and Rivard, 1940). It could be that the season drop in cylinders is factored by the season drop in pH. The data are insufficient for conclusion.

14. *Seniles; Empties; Brokens*

The charts give no adequate evidence that these colony components are subject to season change. A different approach shows a shift in relative contribution of seniles and empties.

Tables 5 to 11 show the completes, buds, cylinders, cones, and brokens comprise a series of decreasing contribution. And that this holds with but few exceptions throughout the months of record despite fluctuations and the season changes in buds, completes, and cylinders.

While the seniles and empties are low men on the totem pole they exchange places as season advances. This is demonstrated by setting up for each month the frequency with which senile percentage is greater, less, and equal to that of the empties, using differences of 10 per cent and over as indicative (Table 16).

This shows that the percentage of seniles exceeds that of the empties with significant frequency in April, May, and June, while it is less with significant frequency in July, August, and September. There

TABLE 16

THE SEASON CHANGE IN FREQUENCY WITH WHICH THE PERCENTAGE OF SENILES IS GREATER, LESS, AND THE SAME AS THAT OF THE EMPTY HYDROTHECAE

Month	April			May			June			July			August			Sept.		
Direction	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=
Senile	5	0	1	15	5	6	15	8	5	10	17	1	8	17	3	5	8	4
Empty	0	5	1	5	15	6	8	15	5	17	10	1	17	8	3	8	5	4

is season shift in relative contribution of these two elements to colony composition.

The empties derive directly from seniles by catabolic dissolution in the normal course of the hydranth life-cycle (Hammett, 1943). Season increase in percentage of empties over that of the seniles is thus evidence of season increase in expression of catabolic activity.

This is perhaps a temperature effect. First, because catabolism is speeded up by temperature rise. Second, because temperature in July, August, and September is higher than in April, May, and June. And third, because an increased progression of seniles to empties has been repeatedly noted in Obelia colonies in laboratory culture on hot days.

Ageing of the colonies may contribute to the shift, since ageing conduces to increased catabolism. These composition data are inadequate for any exposition along this line. The increased destruction here recorded is not factored by season increase in predatory protozoa. The colonies serving for this study were not so infested.

We have now set forth the detectable season changes in contribution of the several hydranth states to Obelia colony composition, and the probable significance of endogenous constitutional, and exogenous season-associated factors thereto. Accessory data help round out the picture.

15. *Variabilities*

The *nature* of the season shift in colony composition has been indicated, i.e., buds decrease and completes increase sharply in May and/or June, after which they follow their respective courses more deliberately. Cylinders gradually decrease, and empty hydrothecae come to take precedence over senile animals during July, August, and September.

The *extent* of change from one month to the next is of interest. This is obtained as follows. The percentage of each hydranth state in each week in all the seven years is summed for each month separately and the average taken. This gives colony composition extant in each month.

What we want to know is not *how* the colony changes from month to month, but *how much*. Index of this is had from the sum of differences between the percentages of one month and those of the next. May buds differ from April by 0.1; cones by 1.1; cylinders by 0.5;

completes by 0.3; seniles by 0.2; empties by 0.5; brokens by 1.1. The sum is 3.8. There was a 3.8 per cent change in composition of colonies in May over that extant in April.

The procedure gives May, 3.8; *June, 9.1;* July, 2.5; *August, 5.9;* September, 1.1. Colony composition changed somewhat in May; markedly in June; slightly in July; considerably in August; negligibly in September.

Four facts emerge: (*a*) The course of season change is a succession of alternating big and little shifts. (*b*) June and August are months of big shift; May, July, and September of little. (*c*) June is the month of greatest change. (*d*) The extent of change, both big and little, decreases as season advances.

Two relations deserve comment, viz., the trend to stabilization of colony composition; and the interruption of this trend in June and August.

The trend to stabilization may be factored by season stabilization of exogenous influences. It may be expression of innate tendency to increasing adjustment of internal activity to external forces. Or it may be expression of Cannonical homeostasis, a *sui generis* internal adjustment to steady state. Growth processes like all others have this property. And colony composition is subtended by growth. Regardless of cause, the trend to attainment and maintenance of colony composition in a steady state is evident.

Marked shift in colony composition is therefore evidence of interfering factors. Since such occurs in June and August it means that factors come into play in these months which differ in nature, intensity, and/or balance from those in play in the immediately preceding months (May, July). The fact that two interruptions of the trend to steady state occur, means either recurrence of one kind of factorial change, or that there are two kinds of factorial change, one peculiar to June and one to August.

The fact that buds are high when salinity is high in early Spring, and rise again somewhat with recurrent rise in salinity in mid- and late Summer is hint of recurrent reaction to recurrent factor.

On the other hand it happens that liberation of matured medusae is at a peak in July. This means that material previously used for substance increase of medusae is now available for hydranth growth.

For there is no particular increase in new gonangia, and the percentage of complete feeding animals is not decreased.

Now the colony is a concinnate assembly of interdependent organisms. It is therefore reasonable to suppose that a shift in utilization and distribution of building material from medusae to hydranths could result in change in colony hydranth composition. The factorial change responsible for the August shift can thus be different from that responsible for the June change. If not in nature, at least in intensity and balance.

The tentative conclusion is that the bi-modal shift in colony composition may be due both to a season recurrent factor (salinity) and to a season shift in balance of endogenous factors (distribution of substance between medusae and hydranths).

Other cyclicism than seasonal is possible. Charts 5 to 11 show recurrent ups and downs in percentages of completes. They seem roughly correlated with the lunar cycle. If they are expression of cyclic reaction to recurrent factors they might be sequalae of differences in composition of water brought in by neap and spring tides. In this connection Burr's (1944) report of possible correlation between lunar change and growth (?) phenomena in trees as elicited by bio-electric potentials in trees are thought-provoking.

Our data as well as those of others show that on any seasonal cyclicism which may exist there may be superimposed a monthly, bi-weekly, and diurnal variation resulting from monthly, bi-weekly, and diurnal change in affecting factors. A particular concatenation of these superimposed cyclic variations could conceivably obliterate here and there any evidence of seasonal. The wonder is not that we get so little evidence of seasonal cyclicism in these Obelia data, but that we get any at all. One reason we get evidence is because the composition of colonies is more determined by endogenous than by exogenous factors.

It may be remembered there is no evidence that temperature is a determining factor in the *course* of season change in colony composition. This is sustained by data on the *extent* of change. Colonies undergo a 9.1 per cent change when temperature rise is 15 per cent in June; but they only undergo a 3.8 per cent change when temperature rise is 19 per cent in May. And they only exhibit a 2.5 per cent change when temperature rise is 9 per cent in July; but they

exhibit a 5.9 per cent change in August when temperature rise is but 1 per cent. There is no correlation between extent of change in colony composition and extent of change in temperature. There is no evidence that change in temperature determines the *extent* of season change in colony composition, any more than there is evidence of its direct influence on the *course* of season change. Again we find constitutional factors dominant.

Analysis would be incomplete without inquiry into season change in variability of each hydranth structure. For this we use the Average Deviation of the monthly Mean. The order of magnitude of one Mean is not necessarily the same as that of others. So the deviations from the several Means are brought to a comparable basis by dividing the Average Deviations by their respective Means. The rationale is this. Consider the numbers as numbers. The May mean for Completes is 67.2 with Average Deviation of 2.28. The May mean for Cones is 4.2 with Average Deviation of 0.58. The percentage deviation of the completes from their Mean is 3.4. That of the Cones is 13.8. Clearly the degree of variation of the cones from their Mean is greater than that of the completes from theirs. And so on similarly. The data are given in Table 17.

The seven hydranth structures fall in the following order of increasing variability; Completes; Buds and/or Cylinders; Cones; Seniles; Empties; Brokens.

It is evident that the processes of maintenance as represented by the complete hydranths are much more stable than the processes of growth as represented by the buds, cones, and cylinders. And it is evident that the processes of growth are much more stable than those

TABLE 17

THE VARIABILITY IN PERCENTAGE CONTRIBUTION OF THE SEVEN HYDRANTH STRUCTURES TO COLONY COMPOSITION TEMPERATURE ALSO AV. DEV./MEAN

Structure	April	May	June	July	August	September
Buds	5.1	10.1	10.4	14.2	11.7	17.6
Cones	7.4	13.8	12.7	15.9	17.1	20.3
Cylinders	8.2	10.1	12.2	12.7	11.4	18.8
Completes	2.8	3.4	3.2	3.9	3.0	4.5
Seniles	12.0	30.0	30.0	34.5	27.5	25.6
Empties	40.0	38.7	31.1	32.7	38.3	28.3
Brokens	25.0	44.2	40.4	38.3	40.0	29.1
Temp. F°	6.0	4.6	4.1	4.2	3.7	3.7

of dissolution as represented by the seniles, empties, and brokens. This ties in with common experience that no matter how growth is distorted, slowed, or brought to standstill, and no matter how disintegration proceeds, maintenance continues to the end (Hammett, 1942, 1943).

These relations suggest that maintenance is less susceptible to distortion by exogenous factors than is growth, and that growth is less affected than dissolution. Be that as it may, the differences are endogenously determined. They are but the external expression of internal controls. They show that the organism is more under control of maintenance than it is of growth, and that it is more subject to growth than it is to dissolution. Conversely they show that the control of the organism over maintenance is greater than its control over growth, while its control over growth is greater than its control over dissolution.

A little reflection makes evident that the generalization evoked by these data is not limited to Obelia, but is applicable to all organisms. Because of this it must be integrated into any holistic concept of organism. It merits extended exposition. But that cannot be done here, for we must get on with our job of analyzing season change in Obelia colony composition.

Looking at the forest instead of the trees Table 17 shows that the products of growth activity (buds, cones, cylinders, completes) increase in variability in late season, while the products of regression and catabolism (seniles, empties, brokens) decrease.

Colony composition is the resultant of balance between growth, maintenance, and regression. There is orderly season shift in this balance. Growth is a larger determinant in early than in mid- or late season. Maintenance is a larger determinant in mid- than in early or late season. And regression is a larger determinant in late than in early or mid-season.

The increased variability in late season of the products of growth can be attributed to the weakened hold of growth on determination of colony composition, since such could condition increased reactivity to interference. And increased variability in late season of maintenance expression (completes) is similarly explained. While the decreased variability in late season of the products of regression can be attributed to the strengthened hold of regression on determination

of colony composition, and a consequent decreased reactivity to interference.

The season shifts in variability are thus by-and-large what could be expected from the season shift in balance of control of colony composition by growth, maintenance, and regression.

The season-associated shift from growth, through maintenance, to regression is colony recapitulation of the hydranth life-cycle. It is a progression common to the ageing of all organisms. There is no reason to suppose it is not here also an expression of normal sequence due to ageing. It is thus not season-determined even though it is season-associated. It is not expression of reaction to season-associated changes in exogenous factors. What such changes do is to accelerate or retard a progression which is inevitable under any and all conditions save those which are completely inhibitive or lethal.

From this it is evident that season does not set the course or sequence of change in organism; only its duration. Sequence is set by organism, and sequence is independent of environment. It follows that organism as organism is superior to environment. Save when this is lethal or completely inhibitive.

16. *Temperature*

That no stone be left unturned we have analyzed the cones, cylinders, seniles, empties, and brokens as we did the buds and completes, with respect to the concordance of their week-to-week direction of change in per cent with the week-to-week direction of change in temperature. The data are in Table 18.

There is good evidence of negative correlation between Cones and Temperature. Of the 126 opportunities cone change is opposite to that of temperature in 43 per cent; the same in but 24. By month-year segregation cone change is opposite in 57 per cent of the 37 combinations; the same in but 22. By period segregation it is opposite in 52 per cent of the 23 combinations; the same in but 22. And negative correlation is the trend in five of the seven years, with positive in but one, and no trend in but one. Of the 18 combinations by months separately, negative correlation is found in 11, positive in three, and no correlation in four.

On the face of it, it would appear as if processes concerned in cone production are held back by temperature rise, and forwarded by tem-

TABLE 18

CONCORDANCE OF WEEK-TO-WEEK CHANGE OF CONE, CYLINDER, SENILE, EMPTY, AND BROKEN PER CENT WITH THAT OF TEMPERATURE (CF. TABLE 15)

	Totals						April			May			June			July			August			Sept.		
	Number			Per cent			S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N
Cones																								
126	30	54	42	24	43	33	4	0	0	5	7	9	3	15	10	7	13	8	10	9	9	1	10	6
Weeks	45	65	16	36	52	13	4	0	0	8	11	2	8	17	3	10	14	4	12	11	5	3	12	2
37	8	21	8	22	57	22	2	0	0	2	3	2	0	7	0	1	5	1	3	3	1	0	3	4
Mth-Yrs	8	18	11	22	49	30	2	0	0	1	3	3	0	4	3	1	4	2	3	2	3	1	5	1
23	5	12	6	22	52	26	3	0	0	1	2	1	0	3	1	0	2	2	1	1	2	0	4	0
Periods	7	11	5	30	48	22	3	0	0	2	2	0	0	2	2	1	2	1	1	1	2	0	4	0
Cylinders																								
126	45	50	31	36	40	25	1	2	1	10	6	5	5	13	10	12	11	5	8	12	8	9	6	2
Weeks	53	60	13	42	48	10	1	2	1	11	8	2	7	16	5	13	14	1	11	13	4	10	7	0
37	15	15	7	41	41	19	1	1	0	4	2	1	1	5	1	3	2	2	2	3	2	4	2	1
Mth-Yrs	14	17	6	38	46	16	1	1	0	4	3	0	1	5	1	1	2	4	3	4	0	4	2	1
23	6	7	10	26	30	44	0	1	2	2	0	2	1	2	1	1	1	2	1	2	1	1	1	2
Periods	5	7	11	22	30	48	0	1	2	1	0	3	1	2	1	1	1	2	1	2	1	1	1	2
Seniles																								
126	46	38	42	37	30	33	1	3	0	8	7	6	11	10	7	12	9	7	8	7	3	6	2	9
Weeks	56	50	20	44	40	16	1	3	0	10	9	2	13	12	3	13	10	5	12	10	6	7	6	4
37	16	10	11	43	27	30	1	1	0	2	2	3	3	1	3	4	2	1	3	3	1	3	1	3
MthYrs	18	14	5	49	38	13	1	1	0	3	3	1	3	1	3	4	2	1	3	4	0	4	3	0
23	10	7	6	44	30	26	0	2	1	2	0	2	1	1	2	2	1	1	2	2	0	3	1	0
Periods	8	5	10	35	22	44	0	2	1	2	0	2	2	1	1	2	1	1	1	0	3	1	1	2
Empties																								
126	44	40	42	35	32	33	0	2	2	10	4	7	11	9	8	8	12	8	9	7	12	6	6	5
Weeks	53	46	27	42	37	21	0	2	2	12	6	3	14	9	5	9	13	6	10	8	10	8	8	1
37	13	14	10	35	38	27	0	2	0	4	1	2	3	2	2	2	4	1	2	1	4	2	4	1
Mth-Yrs	14	13	10	38	35	27	0	2	0	4	1	2	3	0	4	2	4	1	2	2	3	3	4	0
23	8	7	8	35	30	35	0	2	1	2	0	2	2	1	1	0	2	2	2	1	1	2	1	1
Periods	8	6	9	35	26	39	0	2	1	2	0	2	3	1	0	0	2	2	2	1	1	1	0	3
Brokens																								
126	48	37	41	38	29	33	3	1	0	8	6	7	15	7	6	6	10	12	11	5	12	5	8	4
Weeks	56	41	29	44	33	23	3	1	0	10	6	5	16	8	4	8	12	8	12	6	10	7	8	2
37	17	12	8	46	32	22	2	0	0	3	2	2	4	1	2	1	5	1	5	1	1	2	3	2
Mth-Yrs	20	11	6	54	30	16	2	0	0	4	2	1	4	1	2	2	4	1	5	1	1	3	3	1
23	12	8	3	52	35	13	2	1	0	2	1	1	3	1	0	0	2	2	3	1	0	2	2	0
Periods	12	6	5	52	26	22	2	1	0	2	1	1	3	0	1	0	2	2	3	1	0	2	1	1

Top line of all pairs significant: Botton line indicative. S—Same Direction; O—Opposite Direction; N—No Comparison.

perature fall. This is hard to swallow. There is an alternative. Many observations indicate that progress from cone to cylinder may be more rapid than that from bud to cone. Assume both are forwarded by temperature rise. Then in a given time-slice more cones would be sent on to cylinders by a given temperature rise than would be produced from buds. So the percentage of cones would drop. And negative correlation would be recorded.

A like result could come from the processes of cone production from buds being differentially affected by temperature than those of cone progression to cylinders. These matters will be gone into when we come to analyze the growth expressions. All that is here necessary, is to point out that the cone-temperature negative correlation is probably due to an endogenously conditioned growth differential, and is not yet to be taken as evidence that temperature rise retards and temperature fall stimulates processes productive of cones. Things are not always what they seem.

There is no evidence of correlation between cylinder percentage and temperature. So no space will be taken up in detailing the figures.

There is fair evidence of positive correlation between Seniles and Temperature. Senile change is the same in direction as that of temperature in 37 per cent of the 126 opportunities, and opposite in 30. By month-year segregation senile change is the same in 43 per cent of the 37 combinations; opposite in 27. By period segregation it is the same in 44 per cent of the 23 combinations; opposite in 30. And positive correlation is the rule in four of the seven years, with no correlation in one, and negative in two. Of the 18 combinations by months separately, positive correlation is found in eight, negative in two, and no correlation in eight.

This finding is in accord with experience that temperature rise may determine an increase in regression of complete hydranths to the senile state.

There is hint that Empty hydrothecae may be positively correlated with Temperature. Empty change in direction is the same as that of temperature rather more often than it is opposite. But the differences are too narrow for comfort.

There is indication of positive correlation between Brokens and Temperature. Brokens change in direction the same as temperature

in 38 per cent of the 126 opportunities, and in the opposite direction in 29. By month-year segregation broken change is the same in 46 per cent of the 37 combinations; opposite in 32. By period segregation it is the same in 52 per cent of the 23 combinations; opposite in 35. And positive correlation rules in three of the seven years, negative in but one, and no correlation in two. Of the 18 combinations by months separately, positive correlation is found in 11, negative in four, and no correlation in three. Again analysis confirms experience that regression and its products are not indifferent to temperature change.

The fact that the products of regression show positive correlation with temperature, while the products of growth do not, is significant. It confirms by a different approach the already stated assumption that the endogenous processes of growth are paramount in the determination of colony composition and its season change. And it validates the assumption previously expressed that it is not until the hydranths have passed out of the control of the processes of growth that temperature change becomes of signal importance in their contribution to colony composition. Its influence on colony composition, however, is minor, since the products of regression are but a small percentage of the population as a whole.

The student of growth should take note of the fact that although the direction of week-to-week changes in cones, seniles, and brokens is correlated with those of temperature, there is no season change in these colony components like that which occurs in temperature. Which is further evidence that the course of season change in temperature is not the determinant of the course of season change in colony composition.

To recapitulate: although temperature is not the determinant of the course of season change in colony composition, colony components are not indifferent thereto. Its rise above a critical low level releases bud productivity from inhibition; its rise to a critically high level forwards the processes of regression and catabolism. These are the influences which the figures for colony composition bring to record. Other approaches will evoke other relations. But since, save under extreme conditions, colony growth is dominant over colony disintegration, we predict no relations will be found which will overthrow the postulate that the course of season change in colony composition is set by endogenous rather than by exogenous factors.

17. *Illumination*

Certain A.M.-P.M. differences in percentages of hydranths are worth a paragraph or two. To save space and at the same time equilibrate for possible weighting we have condensed the data into monthly indices; one for each of the seven structural states. The estimate is derived from the A.M.-P.M. differences between the averages of the totals of all periods in all years (for the given month); the consistency of direction of difference obtaining in a given month among the seven years; and the consistency of the direction of difference among the periods of a given month (in all years). *M* indicates higher percentage: *F* indicates lower: and = indicates no A.M.-P.M. difference. The A.M. colonies were collected from 4 to 9 o'clock in the morning; the P.M. from 11 A.M. to 3 o'clock in the afternoon.

Table 19 shows that morning colonies tended to have more buds, cones, and cylinders than afternoon. At least in 9 of the 13 instances where a differential existed. Balancing this the percentage of completes was less in the morning than afternoon. At least in 3 of the 4 cases. This is consistent with the earlier demonstration of a negative bud-complete correlation.

Since colony morning composition has more immature members

TABLE 19

THE MORNING-AFTERNOON DIFFERENTIAL IN CONTRIBUTION OF THE SEVERAL HYDRANTH STATES TO COLONY COMPOSITION

Month	Time	Cp	Bd	Co	Cy	Sn	Em	Br
April	A.M.	F	M	M	M	M	F	M
	P.M.	M	F	F	F	F	M	F
May	A.M.	=	F	F	=	M	=	M
	P.M.	=	M	M	=	F	=	=
June	A.M.	M	=	=	F	=	=	=
	P.M.	F	=	=	M	=	=	=
July	A.M.	F	=	M	F	=	M	F
	P.M.	M	=	M	F	=	F	M
August	A.M.	=	=	M	M	F	=	F
	P.M.	=	=	F	F	M	=	M
September	A.M.	F	M	M	M	F	=	F
	P.M.	M	F	F	F	M	=	M

Cp—Completes: Bd—Buds: Co—Cones: Cy—Cylinders: Sn—Seniles: Em—Empties: Br—Brokens.

than afternoon it looks as if growth progression during the night is less than during the day. Consistent with this is the fact that the differential is more general in April and September when the nights are longest, and is reversed in May and June when nights are shortest.

This may be a hint that darkness retards or daylight forwards colony growth progression. On the other hand a morning-afternoon differential in eulittoral sea-water pH has been noted. And the same is probable for temperature and plankton. So no conclusion is possible until more data are available and experiments similar to those of Allard (1943), Bissonette (1944) and others are conducted.

Certain season shifts in A.M.-P.M. differences are suggested. The products of growth (buds, cones, cylinders) contribute more to morning than afternoon colonies in April; less in May and June; and again more in July, August, and September. Balancing this, the maintenance members (complete hydranths) contribute less to morning than afternoon colonies in April; more in June; and less again in July and September.

In other words we have two reversals in direction of difference during the six months period of our study. The fact that these occur in the face of temperature change in one direction leads one to suspect that season change in A.M.-P.M. differential of these colony components is not temperature determined. This fits in with what has gone before.

The time of first reversal (May-June) immediately precedes and extends into the time of greatest shift in colony composition (June). And the time of second reversal (July) immediately precedes the time of second large shift (August). The concordance enhances the probability that June and August are months of deep-seated and particular change in colony composition.

Two products of hydranth regression (seniles and brokens) also show season change in A.M.-P.M. differential. These shift from contributing more to morning than afternoon colonies in April; through an essential sameness in June; to a beginning greater contribution to afternoon colonies in July; which becomes unequivocal in August and September.

Seniles and brokens show positive correlation with temperature change. The late-season shift to afternoon predominance may then be due to the addition of an afternoon increase to an already high temperature level of considerable duration. Which is to say that the

afternoon rise on the existing high temperature level is the extra fillip to hydranth regression for which the months-long temperature increase has been preparative.

We have now exposed about all the season changes in Obelia colony composition which our data make evident. So we come to those exhibited by gonangial population as a determinate product of colony function.

18. *Gonangia*

Two questions want answer. What is the season difference in number of gonangia present in Obelia colonies used in the amino acid studies, and does this give information regarding season differences in gonangia production? And what is the season change exhibited in the number of gonangia at each of the definitive structural states in these colonies, and what does this tell of season differences in gonangial maturation?

McDougall (1943) reports shedding of medusae in Obelia *commissuralis* on April 27, 1941, at Beaufort, N. C., and that the gonangia of most colonies were spent by June 4. He also reports that the colonies had disappeared by mid-June. Whether one could infer from this that viable colonies persisted after the emptying of the gonothecae is a question. Assuming, however, that this is the case, it suggests a season-associated variability in colony production of gonangia. And this, taken with the implication that only some gonangia were mature in April while most were mature six weeks later in June, suggests a season-associated variability in gonangial maturity. A little more precise picture is desirable. Our accumulation is possibly a step in this direction.

As reported earlier, gonangia stem from hydrocaulal anlagen, and increase in size and structural unfoldment up to the stage of completion when the medusae are shed (Hammett, 1935, 1943). The progression exhibits a succession of definitive structural states which is used to identify successive stages in the developmental growth of this product of colony activity. These have been described and arbitrarily designated as the bud, —¼, ¼, ½, ¾, and complete stages. Each may be found in greater or lesser abundance in any adequate collection of colonies. Gonangial growth culminates in the formation of matured medusae and their escape from the gonotheca. A fully

developed gonotheca which is empty, or from which some medusae have escaped, or are escaping from the open distal orifice, is thus a mark of gonangial and/or medusal maturity. And a gonangial bud is a mark of colony gonangial productivity.

On grand average the number of gonangia is somewhat less than one-tenth that of the hydranth. Because of this: because space without profit would be used if each year were recorded separately; and because primary interest is in the overall picture of season change, the data of all years of record have been combined into one set of figures. Those for 1933 are omitted since sure identification of each stage had not then been established. The —¼, and ¼ stages are combined under the ¼ heading. Presentation is thus simplified with no loss of meaning. Table 20 gives the total number of gonangia in each week of record, and the number and average percentage of each structural state in that total. Chart 8 gives visualization of season changes in percentages, and number of gonangia and buds per 10 colonies.

First consider season change in number of gonangia. The number per colony is small, so the number per 10 colonies is used. To get this the number of gonangia was divided by the number of colonies in each week's collection of each year separately and the average taken. The results times 10 are plotted in the chart.

The curve shows low number in early April, peak number in late April, a sharp drop in May, and a slow decline thereafter, with possibly a slight rise in early August.

But it will be remembered that colonies increase in size by addition of new hydranths as season advances. And that it was occasionally necessary in later weeks to remove basal portions in order to bring the colony to a size convenient for growth experiments. It will also be remembered that gonangia inhabit these lower reaches. So the number counted in the retained distal two-thirds to three-fourths of these trimmed specimens may not be that of the whole colonies.

This being so, the curve must not be taken as representing season change in number of gonangia in Obelia colonies as such; but only as record of this in those selected and sometimes cut to size for the amino acid studies.

Despite this limitation, the data have their uses. They show what

TABLE 20

GONANGIAL TOTALS AND AVERAGE PERCENTAGES AT EACH OF THE SEVERAL STAGES OF DEVELOPMENT BY MONTH AND BY PERIODS

Month Period	Numbers						Av. percentages				
	Buds	¼	½	¾	Comp.	Totals	Buds	¼	½	¾	Comp.
April											
1	50	30	0	0	0	80	63	38	0	0	0
2	132	64	3	0	0	199	66	32	2	0	0
3	700	958	361	176	14	2,209	40	41	12	6	1
4	515	1,111	610	131	40	2,407	23	47	23	5	2
Tot: Av.	1,397	2,163	974	307	54	4,895	48	40	9	3	1
May											
1	839	1,211	766	347	141	3,304	22	34	28	13	3
2	684	1,489	791	262	306	3,532	21	41	22	8	8
3	528	1,008	630	335	613	3,114	22	33	24	9	12
4	554	1,112	694	312	357	3,029	20	38	23	10	10
Tot: Av.	2,605	4,820	2,881	1,256	1,417	12,979	21	37	24	10	8
June											
1	637	1,100	734	388	265	3,124	19	37	24	13	7
2	427	917	545	503	946	3,338	13	28	18	17	24
3	417	981	587	586	847	3,418	13	29	18	17	24
4	479	949	654	583	715	3,380	16	28	18	18	21
Tot: Av.	1,960	3,947	2,520	2,060	2,773	13,260	15	31	19	16	19
July											
1	416	946	546	508	1,010	3,426	13	28	16	16	28
2	531	825	463	645	1,212	3,676	17	23	13	18	30
3	436	1,044	657	561	1,004	3,702	13	29	18	15	24
4	411	731	413	542	1.014	3,111	15	26	13	17	30
Tot: Av.	1,794	3,546	2,079	2,256	4,240	13,915	14	26	15	16	28
August											
1	375	844	516	508	931	3,174	13	28	19	15	25
2	336	888	585	444	756	3,009	11	29	18	15	27
3	250	642	423	362	708	2,385	10	26	17	19	29
4	505	1,202	675	351	465	3,198	16	34	22	15	14
Tot: Av.	1,466	3,576	2,199	1,665	2,860	11,766	12	29	19	16	24
September											
1	337	792	458	315	485	2,387	13	30	18	16	23
2	201	594	400	296	532	2,023	11	29	20	16	25
3	43	112	66	54	124	399	11	26	17	13	32
4											
Tot: Av.	581	1,498	924	665	1,141	4,809	12	28	18	15	27
Totals	9,803	19,550	11,577	8,209	12,485	61,624					

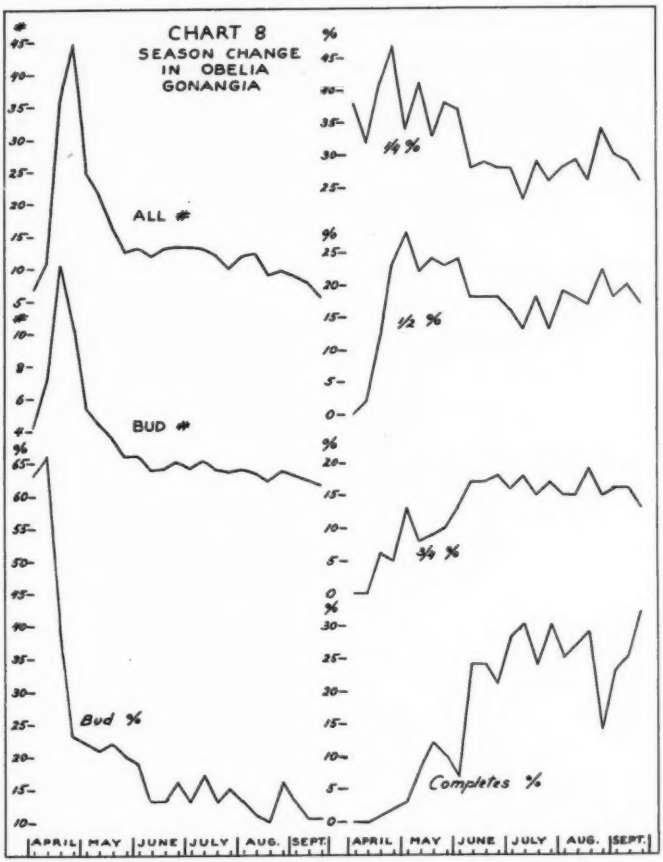
CHART 8
SEASON CHANGE
IN OBELIA
GONANGIA
ALL #
BUD #
Bud %
1/4 %
1/2 %
3/4 %
Completes %
APRIL
MAY
JUNE
JULY
AUG.
SEPT.

CHART 8

may in general be expected of season differences in gonangial availability for growth studies under similar procedure. They are needed for an equilibrated interpretation of growth experiments since the colony is an integrated aggregate in which the growth of one element is related to that of the others. And from them is obtained a fair estimate of season differences in colony gonangia productivity. This is the matter of present interest.

Now even if counts of total gonangia were available throughout the months of record, change therein would not be the best index of the course of season change in colony productivity. For empty gonothecae are lost from time to time without trace or replacement. Hence a decrease in total number of gonangia could be due to this and not to decrease in production of new elements.

The best index is number of buds. For buds are evidence that colonies are active in gonangia production. And the number of buds is mark of the extent the activity is expressed. So difference in number of buds in different weeks throughout a season is index to season change in colony gonangia productivity. The only restriction is that no proper estimate can be had from colonies which do not include the region wherefrom all or most all of the gonangial buds are produced.

In smaller younger colonies gonangial buds commonly appear on the basal hydrocaulus. They are rare here in larger older specimens. In these they stem from somewhat more distal regions. They seem to follow the younger hydrocaulus as this matures distal to the ageing with colony expansion. Apparently the region of colony gonangia productivity which is truly basal in small young colonies, gradually marches up the hydrocaulus, as these grow larger and older.

It can therefore be assumed that the center of gonangia productivity is present in most of the older larger colonies used in our collections of later weeks, despite trimming, just as it is assuredly present in the younger smaller whole colonies used in the earlier weeks. Our material is thus suitable for estimate of season change in colony gonangia productivity.

For this, the number of buds per 10 colonies is used. To get it the average number of gonangia per 10 colonies in each week of record as given on the chart was multiplied by the average percentage of buds as given in Table 20. The result is seen in Chart 8.

It is clear that buds are few in the first two weeks of April, and increase to maximum immediately thereafter.

The small gonangial population of the first week is composed only of buds and ¼s. No ½s, ¾s, or completes are present. The percentage of buds greatly exceeds that of the ¼s. The same holds true in the second week with some small incursion of ½s. The few gonangia of early April are thus largely buds and all immature. This all adds up to the conclusion that early April is a time of low gonangia production.

There is here a difference from hydranth buds. These on the same basis, start off high and then decrease. A fine balance is suggested. Gonangial growth depends on material supplied by feeding hydranths. It could be that gonangia production is held back until enough hydranths evolve from the early splurge in hydranth buds to supply sufficient substance for free and easy gonangial growth. Sustaining is the assumption that colony growth is primarily hydranth increase and only secondarily gonangia production. From which it follows that ingested material may first be allocated to hydranth increase, and only second to gonangial growth.

Alternatively, it could be that the lag in gonangia production as compared with that of the hydranths is a matter of hydrocaulal maturity.

The hydrocaulus grows by distal extension through apical hydranth production. The basal is older because first formed; the apical younger because last produced. The younger has different characteristics than the older (Hammett, 1943). The gradation in this from apex to base is expression of increasing maturity from apex to base. New hydranths for colony expansion spring from immature apical regions of the hydrocaulus. Gonangia stem not from this but from more basal hydrocaulus which is older and more mature. So while hydranth production for colony growth under usual conditions seems to require quite new and immature hydrocaulus, somewhat greater hydrocaulal maturity seems requisite for gonangia production.

As previously noted the colonies of early April are younger than those of later weeks. Their hydrocaulus is of course less mature. At the base as well as throughout. So it could be that gonangia production is held down in the beginning (early April) because it must await attainment of the requisite maturity of the hydrocaulus. The which

only comes as the young colonies increase in size and ageing by the rapid addition of new hydranths characteristic of the period. With this accomplished gonangia production comes to its full expression, and continues to be that concordant with the checks and balances imposed by the maturely constituted colony.

The foregoing is predicated on the assumption that formation of gonangial and hydranth anlagen may be expression of specialization of hydrocaulal cells through endogenously determined progressive intra-molecular rearrangement of cytoplasmic substance (Hammett, 1936, 1943); that a certain stage in this rearrangement must be reached before productivity is possible; and that this stage occurs earlier in the case of hydranth anlagen than in gonangial.

This is all very pretty. But there is another possibility. Formation of gonangial anlagen could be factored by a chemical stimulus specific thereto. This could be a product of hydranth activity. A certain concentration level could be necessary. And hence its production in requisite concentration would require a certain maturity and/or percentage of hydranths in the colony population. On this basis the early April lag in gonangia production could in part be due to low concentration of this purely hypothetical stimulus, and the sharp rise thereafter to its increase resulting from increase in number and maturity of hydranths.

Arguments could be presented for and against these speculations. But this is no place for such intellectual sport. The matter of present importance is that the difference in hydrocaulal site of hydranth and gonangia production is not to be dismissed as just one of those things. As preceding paragraphs show, it involves fundamentals of growth, and exposes our ignorance thereof. Emphasis has been put on the phenomenon in the hope that sometime, somewhere, somebody will come along and so work on these problems we will be freed from the multiplication of words without meaning.

Now back to Chart 8. If the curve of bud number is representative it shows that spring is a time when gonangia production is high; summer a time when it is low. This season difference is like that of hydranth production. It is supported by the fact that the percentage of buds in gonangial population of early weeks is many times that of later.

Explanation of the sharp springtime rise and the subsequent sharp

decline to the summer range must be speculative. No data on causes are available.

The rise could be attributed to increase in specific simulus, as noted above. The decline could be factored by season decrease in hydrocaulal growth. Hydrocaulal extension is a product of apical production of new hydranths. Season decline in the latter naturally involves season decrease in the former. The inevitable consequence would be season decrease in addition of new areas wherein gonangial anlagen could arise. And hence a season decline in gonangia production. Let no one imagine there are no other possibilities. What is done here is to suggest relations indicated by available data. These are inadequate for holistic exposition.

Finally it is noteworthy that colonies in sheltered habitat may produce gonangia from April into September. Though the activity may fall away almost to desuetude it never peters out completely as long as the colonies survive as colonies. So much for gonangia production.

Of next interest is season change in composition of gonangial population. The data have the same limitations and the same usefulness in getting an idea of the nature of season change and other matters as do those for the hydranths (q.v.). The trends are seen Chart 8.

Laying aside particulars for the moment it is evident that the percentage of buds in the gonangial population, and also that of the ¼ and ½ stages, is highest in springtime and lowest in summer, while that of the ¾ and complete stages is lowest in springtime and highest in summer. It is quite evident that the composition of gonangial population changes markedly as season advances.

Gonangia at the bud, ¼, and ½ stages are immature as determined by the state of medusal development and other criteria. Those of the ¾ stage are almost mature. And those at the complete stage have attained maturity.

This being so the gonangial population of springtime colonies is largely composed of immature elements, while that of summer colonies throughout, has more mature elements and more which are mature. Hence the season change in composition of gonangial population is one of shift from immaturity to maturity. Therefore if numbers of immature gonangia are wanted for study, early spring is the time to get them. If mature ones are wanted, summer collections are better.

Once the springtime splurge in production is over and gonangial population reaches its summer range, it seems grossly to settle down to a fairly steady state of asymtotic oscillation. Closer scrutiny however raises the suspicion that even here there may be trends worthy of note if not of decision.

In Chart 8 the percentage of gonangia at the ¼ and ½ stage tends to upturn after the July low. Such is found in five of the six years when each is plotted separately for the ¼ stage: and in all years in the case of the ½ stage. Furthermore a like rise in bud percentage above a previous low is present in five of the six years sometime in July and August. Such however is only shadowed in the bud curves of Chart 8. This is largely because the rise does not occur in the same week in all years, and hence the rise in a given week in one year may be damped in the average value by no rise or even a fall in percentage in that week in some other year.

This suggestion of increase in buds and immature gonangia leads to the suspicion that gonangia production takes a small but significant spurt sometime in July or August.

It is intriguing that this, if real, occurs at about the same time the hydranth buds seem to show a slight season specific increase, and also concurrently or immediately after a sharp season specific rise in percentage of complete hydranths over a previous low. The year-to-year coincidence in these events is striking. Assuming they are related, the increase in hydranth buds would yield more hydrocaulus, and from this additional gonangial anlagen could be produced. And the increase in complete hydranths could yield a higher concentration of our hypothetical stimulus to such production. Either or both could be concerned in the suspected mid-summer increase in gonangia production. It all fits together. Nonetheless it is pure guess work. But guess work based on the incontestable principle that an Obelia colony is an integrated aggregate of interdependent activities of which the changes in relations of the several structures are expressions. We are inclined to believe in the possibility of a mid-summer spurt in gonangia production.

Gonangial population is taken as compounded of gonangia in five definitive structural states. Season change in the relation of each to the population as a whole has been given. Now consider season changes in the relations of the five components to each other. The

base index is the percentage difference between the number of gonangia at one state and the number at another. Thus, Table 20 shows in the first week of April, 50 gonangia as buds, 30 as ¼s. The number of ¼s is 60 per cent that of the buds. The difference is 40 per cent. So the number of ¼s is 40 per cent less than the number of buds. And the contribution of ¼s to gonangial population in the first week of April is 40 per cent less than that of the buds. And so on similarly for each week of record, and each structural twosome. The data are in Charts 9 and 10. When the values are below the zero line, the contribution to gonangial population of the first designated structure is less than that of the second. When they are above, the contribution of the first is greater than the second. The following season shifts in interstructural relations are exhibited.

Gonangia of the ¼ stage contribute less than those of the bud in early April, but from then on they contribute more, and increasingly as season advances.

Gonangia at the ½ stage contribute less than those at the bud in early April, but their contribution increases sharply and soon comes to be greater than that of the buds, and increasingly greater as season advances.

Gonangia at the ¾ stage contribute less than those at the bud in April, May, and mid-June, but their contribution increases during these weeks, and becomes greater than that of the buds from mid-June on.

Gonangia at the complete stage contribute less than those of the bud in April, May, and early June, but their contribution increases during these weeks to become greater than that of the buds from mid-June on, and increasingly.

The ½ stage gonangia contribute less than ¼ stage throughout the months of record, but their contribution rises towards that of the ¼s during April and stays there.

The ¾ stage gonangia contribute less than the ¼ stage throughout the months of record, but their contribution rises towards that of the ¼s from April to July and stays there.

The contribution of complete gonangia is less than that of the ¼ stage during April, May, and June, but it increases during these months to become greater than that of the ¼s during July, after which it again becomes less in general.

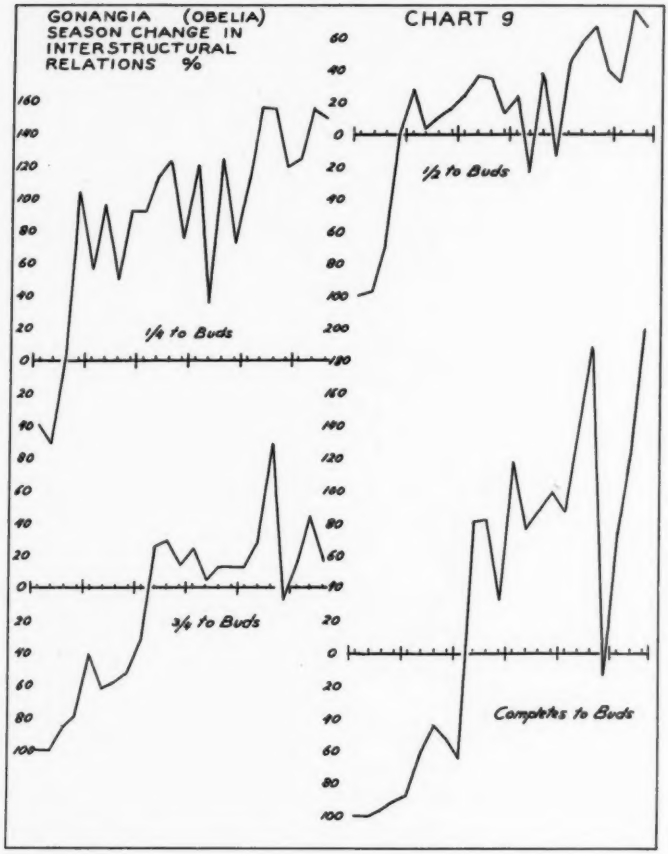
GONANGIA (OBELIA)
SEASON CHANGE IN
INTERSTRUCTURAL
RELATIONS %
CHART 9
1/4 to Buds
1/2 to Buds
3/4 to Buds
Completes to Buds

CHART 9

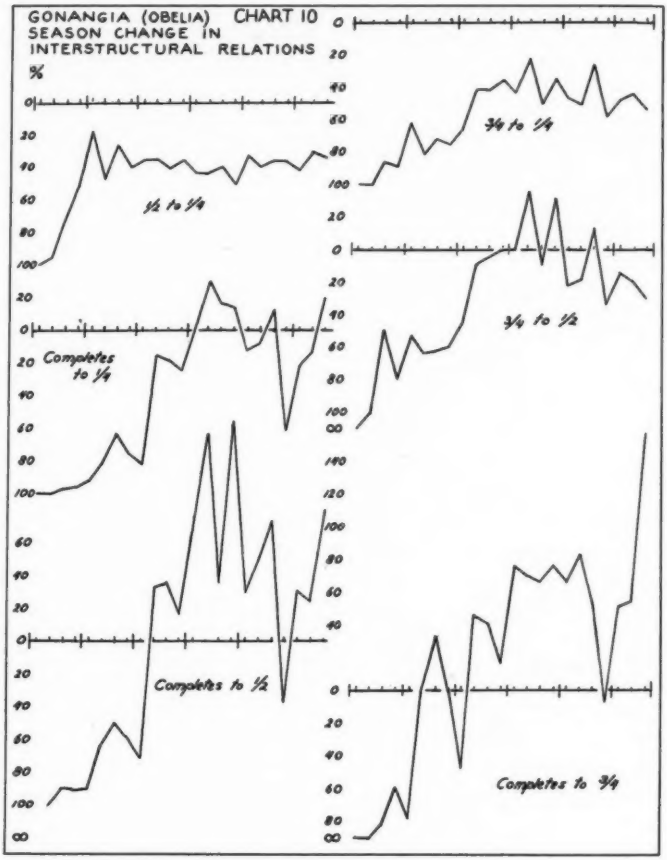
GONANGIA (OBELIA) CHART 10
SEASON CHANGE IN
INTERSTRUCTURAL RELATIONS
%
½ to ¼
¾ to ¼
Completes to ¼
¾ to ½
Completes to ½
Completes to ¾

CHART 10

Gonangia at the ¾ stage contribute less than those at the ½ during April, May, and most of June, but increases during these months to become greater than that of the ½ stage in July, after which it again becomes less in general.

The contribution of complete gonangia is less than that of the ½ stage during April, May, and early June, but it increases during these weeks to become greater than that of the ½s from then on.

Gonangia at the complete stage contribute less to gonangial population than do those of the ¾s in April and early May, but the contribution increases during these weeks to become in general greater than that of the ¾s in later season, and apparently increasingly.

Though the multiplicity of season shifts in interstructural relations and the differences in timing of the change from a lesser to a greater contribution is perhaps confusing to the uninitiated, the picture is actually one of an ordered pattern and fine consistency.

If just one fact gets firmly fixed in mind, resolution of the data into coherency is inevitable. This fact is that the buds, ¼s, ½s, ¾s and completes comprise a structural series of successive stages in gonangial growth. So that as we go from buds to ¼s; from ¼s to ½s; from ½s to ¾s; and from ¾s to completes, we are dealing with progressively more developed gonangia.

So then when we find as we do here, that as season advances, the ¼s come to contribute more to gonangial population than the less developed buds; that the contribution of the ½s rises towards that of the less developed ¼s; that the ¾s come to contribute more than the less developed ½s; and that the contribution of fully developed gonangia also comes to be more than that of the lesser developed ¾s; when, in fact, we see that as season advances each more developed state becomes, or tends to become, a greater contributor to gonangial population than its less developed antecedent, we get a much more elegant demonstration than that previously given, of the fact that the gonangial population of later weeks, has more mature members than that of earlier weeks.

Season differences in the coming into superiority of the more advanced stages over the less advanced are worthy of note. In general, the more mature the gonangium, the later it becomes dominant. Charts 9 and 10 show that the ¼s pass the buds in April; the ½s pass the ¼s in May; and the ¾s pass the ½s in July. Chart 9

shows the ¼s pass the buds in the 3rd week of April; the ½s do this the 1st week of May; while the ¾s and completes come along over the 2nd week of June. Chart 10 shows that the ½s rise closest to the ¼s early in May; while the ¾s do not attain their asymptote with the ¼s until the last of June; and the completes do not pass the ¼s until the 3rd week of July.

So in three different comparisons nine of the 12 possibilities show consistency in relation between stage of development and coming into prominence of the more advanced state.

There are three inconsistencies. The ¾s pass the ½s later instead of earlier than the completes pass the ¾s. The ¾s pass the buds concomitantly with instead of earlier than the completes. And the ¾s pass the ½s later instead of earlier than the completes become superior thereto.

These divergencies from ordered progression are essentially a matter of out of placeness of the ¾s in relation to the completes. Our guess is that the rate of growth from the ¾ to the complete state is a factor. This is probably different from that of bud to ¼; ¼ to ½; and ½ to ¾. It will be gone into in a later report.

Despite these inconsistencies the evidence is preponderantly to the effect that the more mature the stage of development, the later its contribution to gonangial population becomes, or tends to become, greater than that of its antecedents. This simple fact that the more advanced stages take over later than the less advanced means that as week follows week the quality of contribution progressively increases in maturity. The seriation is thus supplementary evidence that gonangial population of later weeks is more mature than that of earlier.

Now the function of gonangial growth is the production of medusae capable of emerging from the gonotheca under their own power, and of existing thereafter as independent entities.

So just as we are interested in knowing the course of season change in gonangia production, and the course of season change in maturity of gonangial population, so are we interested in knowing the course of season change in medusal maturation and/or production of free-living medusae.

For this the complete gonangium is used. Completeness is marked by a patent distal orifice from which the within-contained medusae are about to emerge, are emerging, or have emerged in their entirety.

Season difference in number of such is the index of season difference in medusal maturation and/or production of free-living medusae. Our data give two approaches; one through season change in percentage of complete gonangia in gonangial population; the other through season change in number of complete gonangia per 100 colonies.

Consider the first as shown in Chart 8. Three phases are discernible. A beginning phase when no free-living medusae are produced. An intermediate phase of relatively low production. And a succedent phase of relatively high production.

The phase of no-production is short and confined to colonies collected in the first two weeks of April.

The phase of relatively low production begins with the third week of April and extends into the first week of June. During this phase there is general trend to increasing production of free-living medusae.

The phase of relatively high production, which is the third and final phase, begins with the second week of June, which is the beginning of summer, and extends to the end of record in the third week of September. The transition from low to high productivity is abrupt and the percentage is doubled. There is general trend to increase in the beginning. The peak occurs in July. This is followed by a trend to recession, but this never reaches the level of the low production phase. The record ends with rise to a final high.

The same three phases are shown in the week-by-week number of complete gonangia per 100 colonies. The data are obtained by procedure similar to that used in getting the number of buds per 10 colonies (q.v.). They are: April—0, 0, 4, 9; May—7, 17, 20, 13; June—9, 29, 31, 28; July—*37, 39,* 30, 30; August—30, 33, 26, 13; September—21, 20, 18. According to this analysis medusal maturation and production of free-living gonangia is greatest during the first two weeks of July.

The overall picture then is that medusal maturation in *Obelia geniculata* in this region is a season-associated variable, and that its course is one of no or little production of free-living organisms in early spring; a low but rising production in late spring; and a high production throughout the summer, with greatest activity in July, possibly in the first two weeks, and some recession thereafter.

A comparison with the report of McDougall (1943) is instructive. He found plenty of *Obelia commissuralis* at Beaufort, N. C., in early

April but no shedding of medusae until the 27th. We find plenty of *Obelia geniculata* at Provincetown in early April; but no empty or emptying gonangia until the third week in April. The agreement, regardless of difference in species, region, and habitat, is good evidence early spring is not a time of medusal maturation in Obelia.

McDougall also records that "By June 4 the gonosomes of most colonies were spent—." Here we differ, for 78 per cent, or the large majority of the *geniculata* gonangia at Provincetown were far from maturity at this time, and only 10 per cent had produced free-living medusae, and only 12 per cent were even close thereto.

No one would question that this difference is factored by species difference and difference in temperature between Beaufort and Provincetown. But McDougall's observations were of colonies growing exposed on pilings, while ours were of colonies growing in sheltered habitat. And the former disappear by early summer because of desiccation through wind, sun, and salt, while the latter persist into September and beyond because protected from these destructive influences. Furthermore, we have noted that immature gonothecae (Hammett, 1943) are found empty in colonies disintegrating from exposure while none such are found in colonies from protected habitats. Since McDougall gives no evidence of having noted the structural distinction between immature and mature gonothecae, it could be that the empty structures noted by him in June were as often the product of destructive factors as of medusal maturation. Thus difference in habitat is undoubtedly a factor in the difference obtaining between our records and those of McDougall, and the inference that early June is a time of medusal maturation at Beaufort must be taken *con grano salis*.

In 1933 Grave published a report on the *"Rate of growth, age at sexual maturity, and duration of life of certain sessile organisms at Woods Hole, Mass.,"* which includes data on *Obelia commissuralis* in the Eel Pond of that community. The data and comments, despite the title, are such that no information on season progress in gonangia production and medusal maturation may be gleaned therefrom. And the records of colony growth only show that colonies do grow. While the life-duration data are stultified by the character of the habitat. It would have been nice to have had data from Woods Hole to compare with those from North Truro.

From all this it is evident that somewhat more precise data than those given by McDougall and Grave are necessary if even a fair approximation to knowledge of season change is to be had. Helpful is recognition of definitive structural states in gonangial growth; counts of each from time to time; and the use of colonies from protected rather than exposed habitats.

Now there is fundamental difference between gonangia and hydranths with respect to season shift. There is no general season change in the order in which the hydranth variates contribute to hydranth population. But there is definite season change in the order in which the gonangial variates contribute to gonangial population. This means that season progress of gonangia production and growth differs from that of the hydranths.

Such should of course be expected from the fact that the chemical processes responsible for hydranth growth differ from those responsible for gonangial. Evidence is had not only in the structural differences exhibited during growth, but also in those extant in the completed products. And further evidence is had in the fact that the function of hydranth growth differs from that of gonangial. The former leads to colony growth and maintenance. The latter to medusae production and species continuance.

One set of chemical processes produces a complex chitinized structure which remains with the colony and contributes to its nourishment. The other set produces a blob of jelly which swims away to live by itself and give rise to the beginning of another colony.

The reaction chains are different. It would be strange indeed if the season shift in numerical relation between the successive products thereof did not also differ. The fact that the two sets of variates do differ so strikingly is but convenient mathematical proof of what *a priori* reasoning tells must happen under the circumstances.

These several data all add up to demonstrate the intercalation of season change in composition of gonangial population with that of hydranth. Although each is primarily set by factors peculiar and specific to each, the expression of neither is completely independent of that of the other. In the first place season change in new hydranth production is basic to season change in gonangia production, since it determines season progress in formation of hydrocaulal regions wherefrom gonangial anlagen are produced. In the second place sea-

son increase in mass of gonangial substance derives from season increase in material made available by season increase in feeding hydranths. And finally the recession of medusal maturation from the July peak, releases material for hydranth growth, which well may be a factor in the later rise in percentage of complete hydranths from a previous low. The data are evidence of interdependency. They indicate that season change in composition of gonangial population is the more dependent variable.

D. Extrapolation

It has been stated that: Science is the accumulation and application of data on the relations between things; between states; and between events: and on relations between things, states, and events (Hammett, 1941).

It is therefore pertinent to consider how this highly technical and detailed accumulation of data on season change in Obelia colony composition may be related to expression of season change in organisms in general.

It is strikingly apparent that the course of season change in budding activity in Obelia is like that exhibited by leaf budding in plants in the same region. In both, budding activity is high in spring; low in summer.

Budding in Obelia is at bottom the same as budding in plants. In both, budding is a distinctive and characterizing growth activity wherefrom elements of like basic function are produced. The hydranths and the leaves are both organs whereby material is supplied for growth and maintenance. In both Obelia and plants, buds spring from newly constituted anlagen cells. In both, buds spring from cells reconstituted after injury. And in both, buds spring from cell rests left by the normal passing away of a pre-existing element.

Furthermore, budding is subtended by the growth activity of initiation (Hammett, 1943). And expression of initiation depends on chemical processes specific and peculiar to the activity. These are common to initiation wherever it is expressed. And those specific and peculiar to a type of initiation expression are common to that type wherever it is expressed (Hammett, 1936, 1942, 1943). So, since the same types of bud initiation are expressed in Obelia as in plants, it is clear that bud production is subtended by the same chemi-

cal processes in both. This then is the base in which the similarity in season change in budding in Obelia and plants is grounded.

It is conceivable that the annual springtime recurrence of gonadal hyperplasia in birds as reported by Riddle (1925, 1926) has somewhere in it the same factor. Indeed burgeoning of growth in spring is a phenomenon common to most organisms, and is a commonly exhibited season-associated expression and/or sequence of the growth activity of initiation. Furthermore, the springtime high is generally followed by a summer low. Thus through season change in expression of initiation, the which is mediated through chemical processes common to all initiation and specific and peculiar thereto, is season change in Obelia colony composition related to season change in organisms in general.

Now season changes in organisms are customarily attributed to season changes in external factors such as longer or shorter days, more or less ultraviolet light, more or less heat, and their concomitants (Riddle, 1938). But our data show that some season-associated changes are endogenously determined.

The spring-summer differential in Obelia budding is definitely due to the natural developmental progression whereby the dominance of growth activities comes to be superseded by that of maintenance. Such is also responsible for the shift from high springtime to low summer leaf-budding in plants. Indeed it is probable that all the spring-summer initiation differentials exhibited by organisms are similarly constituted since all growing organisms exhibit shift from growth to maintenance dominance.

Furthermore, since the spring-summer changes in initiation are endogenously determined, and since initiation is the beginning of functional unfoldment, it is probable that many season-associated changes in functional activity also are endogenously determined. The change from the springtime enthusiasm for mating to the summer weakening exhibited by many animal species is an example.

Finally the possibility should not be neglected that some of the long periodic swings are endogenously determined by chemical processes analogous to those which give rise to Liesegang rings (d'Arcy Thompson, 1938), and processes analogous to those concerned in Cannonical homeostasis. The annual springtime recurrence of brilliant plumage in male birds may be in part expression of the

former. The opposite season-associated changes in organ weights in doves (Riddle, 1925, 1938) may be expression of the latter.

The obverse of the fact that many season-associated changes are endogenously determined is the fact that constitutional relations may also determine an out-of-season expression of what is ordinarily a season-associated activity.

Thus it sometimes happens that Obelia colonies are stripped of their complete hydranths by a July, August, or September storm, and left as ordered aggregates of bare and broken pedicels. Such colonies do not die, but immediately proceed to put forth a multitude of buds wherefrom the lost elements are replaced.

In plant life similarly. The mid-September hurricane of 1944 stripped trees, shrubs, and bushes of Cape Cod of their foliage. Yet, despite the lateness of the season, twigs and branches budded profusely into new green leaves within the week.

These out-of-season resurgencies in budding were not determined by season change, but by change in constitutional relations set into play by injury. Hence, just because an activity is season-associated is no evidence it is season-dependent.

All these facts and their cognate derivatives lead ineluctably to the conclusion that some modification of the customary allocation of season changes in organisms exclusively to exogenous factors is in order. Every discussion and interpretation should recognize and include consideration of the significant, ubiquitous, and inevitable participation of endogenous factors. Otherwise it is non-integrative and comparable to a cart with one wheel missing. To state that seasons dominate life and activities is to state a half-truth. And half-truths are the perquisites of politicians; not of scientists.

The annual recurrence of similar phenomena at similar times engendered in the mind of early man the concept of season change as cause and season-associated phenomena as effect. As a result throughout the ages and even into the present, concern with external factors has overwhelmed and smothered attention to internal. And this despite the vast accumulation of evidence that the organism itself has cyclic propensities. Thus the pertinacity of tradition has in this field as in many others obstructed that attention to alternative hypotheses without which no interpretation ever comes to well-rounded fruition. We too might have been bogged in tradition if the data had not pried us out.

E. Summary and Conclusion

An analysis is given of season change in composition of Obelia colonies collected from protected habitats in Provincetown Harbor over the months from April or May into September during the seven years from 1933 to 1939.

The change in relative number of hydranths at each of the seven definitive stages in the hydranth life-cycle as season advances is taken as the measure of season change in colony composition. For this there was record of 896,302 hydranths on 53,056 colonies.

Supplementing this a similar analysis is made of season change in composition of the gonangial population in the 1934-1939 colonies. Here six definitive stages as expressed by 61,624 gonangia were used.

Also given are concomitant local records of atmospheric (F°) temperature for all years; impressions of season changes in plankton and salinity; reference to day length data as given in the *Old Farmer's Almanac;* and morning, mid-day, and early evening pH records from June 13 to September 10, 1941, of local eulittoral sea-water. These latter showed for the single year of observation, a day-time increase in alkalinity, a low-high-tide differential, and a gradually decreasing alkalinity over the months of record.

The chief season-associated changes exhibited by Obelia colonies from April into September are as follows:

Hydranth buds decrease and complete feeding animals increase.

Gonangial buds and immature gonangia decrease, while almost mature, and medusa-shedding gonangia increase.

Peak budding is in early spring. Peak attainment of complete hydranths in August. And peak production of free-living medusae in July.

The composition of hydranth population, and that of gonangial also, progresses from a state of lesser to a state of greater maturity over the months from April into September.

The data give no evidence that this progression is determined by season change in temperature, illumination, pH, or plankton. And why should it be? Progression towards maturity is a property of all organisms as they grow. It is expressed in uncounted myriads regardless of season. It is the outward expression of an inner determined chain of chemical reactions which leads to the development of

a maturely functionable organism. Its rate and its timing may be affected by season change in exogenous factors, but its sequence is exclusively a matter of endogenous direction.

The only possible conclusion with regard to the course of season change in Obelia colony composition over the months from April into September is that this is not determined by concomitant season changes in exogenous factors, but is determined by the reaction chain of endogenous chemical processes responsible for developmental growth.

Application of this principle is not restricted to Obelia colonies. It is similarly in force in leaf-budding. And it well may be of considerable significance to many season-associated changes in many organisms, which are customarily attributed to domination of season change in exogenous factors. "The fate of all explanation is to close one door only to have another fly wide open" (Fort, 1941).

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